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Comparison of defence buzzes in hoverflies and buzz-pollinating bees

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Running title: Defence buzzes in hoverflies and bees

Abstract

Bees and many flies, particularly hoverflies (Syrphidae), have evolved a diverse range of mechanisms to gather pollen from a wide variety of flowering plants. Bees and hoverflies use protein-rich pollen as a food resource to mature reproductive organs and eggs and, in bees, to feed their larvae. A particularly striking pollen-collecting behaviour involves the production of thoracic vibrations to dislodge pollen from flowers. Vibratile pollen collection is widespread in bees (>11,600 species) but extremely rare in flies (~1 species of hoverfly). Why the use of floral vibrations to collect pollen is so rare among flies is currently unknown. A hypothesis proposed to explain why flies do not engage in vibratile or buzz pollination is that they are unable to reach the vibration amplitude required to expel pollen from anthers. Here we document, for the first time, the mechanical properties of non-flight thoracic vibrations produced by hoverflies and compare them to the vibrations produced by buzz-pollinating bees under similar contexts (defence buzzes). We analysed ~4,000 vibrations produced by nearly 300 individuals representing 20 species of hoverflies and 22 bee taxa, recorded using a miniature piezoelectric accelerometer. We characterised both frequency and acceleration amplitude components of non-flight thoracic vibrations and their relationship to insect size. Our results show that, after accounting for size, buzz-pollinating bees and hoverflies produce vibrations with similar acceleration. We show experimentally that the acceleration amplitude produced by some hoverflies is sufficient to elicit pollen release from buzz-pollinated flowers (*Solanum dulcamara* and *S. rostratum*). Our study does not support the hypothesis that the dearth of buzz-pollinating flies is caused by their inability to produce vibrations of sufficient amplitude. We discuss alternative hypotheses to explain why most flies do not engage in buzz pollination and suggest that the lack of buzz-pollinating flies might be best explained through their life history.

Keywords: Bees, behaviour, biomechanics, buzz pollination, flies, Syrphidae.

Introduction

In addition to collecting nectar and other floral resources, bees (Hymenoptera: Apoidea: Anthophila) and some flies (Diptera) visit a wide diversity of flowering plants to gather pollen (Thorp 2000, Woodcock *et al.* 2014, Cook *et al.* 2020). Pollen consumption is known in fly species in the families Bombyliidae, Empididae, Muscidae, Tabanidae, Ceratopogonidae, and particularly Syrphidae (Inouye *et al.* 2015). Pollen is an essential resource for bees and hoverflies (Syrphidae), because the nutritious, protein-rich pollen grains are used to support development of sexual organs and to build nitrogen reserves in the eggs (Kevan & Baker 1983, Thorp 2000, Larson *et al.* 2001). In flies, pollen consumption mostly occurs in the adult stage, although in some flower-brooding hoverflies, larvae can also feed on pollen (Holloway 1976, Nunes-Silva *et al.* 2010, Inouye *et al.* 2015). However in bees, pollen is needed to feed larvae in both solitary and social species (Thorp 2000). Thus, more than 6,000 species of hoverflies (McAlister 2017) and 20,000 species of bees (Danforth *et al.* 2019) depend heavily on pollen collection.

Bees and hoverflies have evolved a distinct set of structural and behavioural adaptations to extract pollen from flowers. In bees, specialised structures to collect pollen include hooked hairs on legs and mouthparts, or clusters of hairs on the top of the head (Thorp 1979, 2000). Moreover, the majority have external structures specialised for pollen transport (Thorp 2000), including modified hairs forming corbiculae in legs (e.g. Apidae) or scopae in legs or other body parts (Thorp 2000). Structural adaptations for pollen collection in flies are less well-studied. Examples include the presence of branched, plumose, or curly-tipped body hairs that help pollen to adhere to the body. More rarely, some hoverflies also use bristles arranged in combs in their legs to aid with pollen collection (Holloway 1976, Larson *et al.* 2001), and males of *Platycheirus spp.* use flattened tarsi to squeeze pollen out of anthers (Inouye *et al.* 2015). In contrast to bees, hoverflies do not need to transport large amounts of pollen as they consume it directly from the anther or from their bodies during floral visitation (Larson *et al.* 2001).

As well as structural differences, there are also behavioural differences associated with pollen collection between bees and flies. Pollen collection in bees involves scraping, rubbing, tapping and rasping with their legs or other body parts (Thorp 2000, Russell *et al.* 2017, Konzmann *et al.* 2019, Portman *et al.* 2019). Hoverflies use pollen grooming and scraping routines, either while hovering or resting, to collect and consume pollen accumulated in or actively transferred to their front legs (Holloway 1976). A particularly striking behavioural difference among pollen-collecting bees and hoverflies is their ability to produce vibrations to remove pollen from flowers, also known as floral vibrations or sonication (Rick 1950, Michener 1962, Buchmann *et al.* 1977, Vallejo-Marin

2019). While the behaviour of producing floral vibrations has evolved more than 40 separate times in 58% of the 20,000 species of bees (Cardinal *et al.* 2018), very few of the 6,000 species of hoverflies are known to use vibrations to collect pollen from flowers (Figure 1). The only published exception is the Mexican cactus fly, *Copestylum mexicanum* (formerly *Volucella mexicana*), which vibrates *Solanum douglasii* (Buchmann *et al.* 1977). Why the use of floral vibrations to collect pollen is so rare among flies is currently unknown.

Floral vibrations are one of several types of non-flight thoracic vibrations produced by bees and flies across different behavioural contexts (Buchmann 1983, Hrncir *et al.* 2005, Rashed *et al.* 2009). Non-flight thoracic vibrations are produced by the same asynchronous muscles that power flight in both bees and flies (King *et al.* 1996, Dickinson *et al.* 1998, Pritchard & Vallejo-Marín 2020b). Social bees in the genera *Apis* and *Melipona* use thoracic vibrations to communicate with nestmates (Hrncir *et al.* 2005, Hrncir *et al.* 2011), while males of the solitary bee *Osmia bicornis* use vibrations to entice females to mate (Conrad & Ayasse 2015). Moreover, bees and hoverflies produce non-flight thoracic vibrations under duress as an alarm, warning or deterrence signal. In bees, these "defence buzzes" serve as an alarm or aposematic signal to deter potential predators (Kirchner & Röschard 1999). Hoverflies also produce defence buzzes, and it has been suggested that they represent either a general alarm signal or acoustic mimicry with bees (Rashed *et al.* 2009, Moore & Hassall 2016).

One hypothesis that could explain why flies do not use vibrations on flowers is that they are unable to reach the vibration amplitude required to expel pollen from anthers. Although bees apply vibrations to remove pollen from many different types of flowers, the use of floral vibrations is strongly associated with buzz-pollinated "poricidal flowers", flowers in which pollen is concealed in structures, usually made of enlarged anthers, that open only through small apertures or pores (Buchmann 1983, De Luca & Vallejo-Marin 2013, Russell *et al.* 2017). Theoretical and experimental studies of buzz-pollinated flowers, show that the amplitude of the vibration (measured as acceleration, velocity or displacement) determines pollen release (Buchmann & Hurley 1978, De Luca *et al.* 2013, Rosi-Denadai *et al.* 2020). King & Buchmann (2003) proposed the hypothesis that an inability to reach the required acceleration amplitude could explain why some bees, such as *Apis mellifera*, do not employ vibrations to remove pollen from poricidal flowers. This hypothesis could be extended to other floral visitors including flies, generating the prediction that hoverflies should generally achieve lower acceleration amplitudes than buzz-pollinating bees.

Since most hoverflies do not produce floral vibrations, addressing this hypothesis requires the comparison of other types of non-flight thoracic vibrations. Defence vibrations provide such an

opportunity as they occur across taxa and can be experimentally induced in both bees and hoverflies (Rashed *et al.* 2009, De Luca *et al.* 2014, Moore & Hassall 2016, Pritchard & Vallejo-Marín 2020b). However, when using defence vibrations to test hypotheses related to pollen release from flowers, it is important to consider that vibrations across different behaviours have different properties (Hrncir *et al.* 2005). The properties of defence vibrations compared to floral vibrations have only been studied in a handful of bee species. Use of non-contact laser vibrometry to measure vibrations directly on the thorax of *B. terrestris ssp. audax* shows that floral vibrations have higher frequencies than defence vibrations (313 ± 3 vs. 236 ± 4Hz), and larger peak acceleration amplitude (518 ± 19 vs 297 ± 12ms^{-2}) (Pritchard & Vallejo-Marín 2020b). Therefore, defence vibrations may provide a conservative estimate of the acceleration amplitude produced during floral vibrations.

Here, we compare defence vibrations produced by different species of buzz-pollinating bees and hoverflies and determine their capacity to release pollen from buzz-pollinated flowers. We address three main questions: (1) What is the relationship between insect size and peak acceleration of non-flight thoracic vibrations? Previous work has shown that within a single bumblebee species (*Bombus terrestris*), individual size is positively correlated with peak velocity (De Luca *et al.* 2013). We therefore hypothesise that both bees and hoverflies show a positive relationship between size and peak amplitude acceleration. (2) After controlling for individual size, do bees and hoverflies differ in the peak acceleration amplitude achieved during non-flight vibrations? We predict that after accounting for individual size, hoverflies should achieve lower peak acceleration amplitudes than bees (Hymenoptera). (3) Can defence vibrations reach the acceleration required to release pollen from buzz-pollinated flowers? We experimentally determined pollen release in two species of buzz-pollinated flowers subject to defence vibrations by both bees and flies.

Materials and Methods

Specimen collection

We collected bees and hoverflies in the summer of 2020 (May-August) in three geographic regions in Scotland: Stirlingshire, the Orkney Isles and the Outer Hebrides. We visited flower-rich sites and collected insects visiting flowers or in surrounding vegetation using insect nets or plastic vials. For each specimen we recorded the location and plant species when collected on flowers. Immediately after capture, insects were placed into individual plastic vials, labelled and stored in a cooler with ice packs for transport. We measured thoracic vibrations of each insect in an indoor lab as soon as possible following collection, usually within 3hrs.

Data acquisition

Because we were interested in determining the capacity of insects to transduce vibrations, we measured thoracic vibrations applied by insects onto a calibrated miniature uniaxial piezoelectric accelerometer (0.2 g; 352C23, PCB Piezotronics, Hückelhoven, Germany). The experimental system consisted of the 0.2 g miniature accelerometer attached to the end of a split bamboo flower stick (3.7mm diameter x 200mm length, LBS Horticulture, Colne, Lancashire) by 30mm of connecting electrical cable (1mm diameter, PCB Piezotronics) between the base of the accelerometer and the end of the stick. The cable was attached to the stick with tape (Scotch 810 Magic™Tape, 3M). The stick was held in place by attaching it to a small plastic container through two small holes (Figure 2). Empirical measurement of the vibrational properties of the experimental system indicated a resonant frequency of approximately 17Hz (data not shown) and thus below the measured insect vibrations. We did not observe any significant contribution of resonance to the vibrations analysed.

Vibrational data was acquired with a C-Series Sound and Vibration input module with 24-bit resolution (9250; NI, Newbury, UK) on a Compact DAQ chassis (cDAQ-9171, NI) connected to a portable computer (Elitebook 850 G5, HP Inc, Glasgow, UK) through a USB port. We used custom-made software written in LabView NXG 5.0 (NI) for signal conditioning and data acquisitions at a sampling rate of 10,240Hz. The data were stored in the computer as TDMS files (NI high throughput file format) to reduce buffer size and preserve acquisition information, and subsequently converted to tab-separated text files for downstream analyses using custom software.

Acquisition of thoracic vibrations

To measure the vibrations transduced by insects onto the accelerometer system, we tethered the chilled insects using a loop placed between head and thorax, made of nylon thread (0.18 mm diameter) held at the tip of a metal syringe needle (1.24mm x 13mm) with a blunted end (Pritchard & Vallejo-Marín 2020b). In some cases, further chilling was required where insects were still very active and we achieved this by briefly placing them in a freezer (-20°C) until the insect became inactive (1-3 minutes). The tethered insects were then allowed to return to room temperature. Once the insect had fully recovered, we held the base of the syringe needle by hand and gently but firmly pressed the insect's thorax (dorsal surface) against the accelerometer along the axis of vibration measurement of the accelerometer. The insect often begun producing defence vibrations after being pressed against the accelerometer. In some cases, we also induced the production of vibrations by breathing onto the insect. We aimed to record approximately 45 seconds for each insect but the length of the recording varied across individuals. After recording, the insects were freeze-killed by placing then at -20°C overnight.

Insect identification and estimate of thorax size

Insects were pinned, air dried, and identified with the help of taxonomic and field keys (Stubbs & Falk 2002, Ball & Morris 2015, Falk & Lewington 2015). To obtain an estimate of thorax size across bees and hoverflies, we used thorax width measured at the widest point with a digital calliper (0.01mm precision; CD-6"CSX, Mitutoyo Inc, Japan). In bees, body mass and intertegular distance are positively correlated (Cane 1987), and thorax width should be positively correlated with intertegular distance. We chose this measure in flies as well in order to (1) obtain a trait directly comparable trait between bees and flies, and (2) because we were interested in the vibrations produced by the thoracic muscles, which are responsible for both powering flight and for producing defence and floral vibrations (Pritchard & Vallejo-Marín 2020a).

Pollen release

We conducted a small experiment to qualitatively determine the capacity of hoverflies to remove pollen from buzz-pollinated flowers with poricidal anthers. We used two species of nectarless, buzz-pollinated plants for this experiment. The first was *Solanum dulcamara,* a perennial plant native to the British Isles with small flowers (15mm across) and five anthers of similar size (4-5mm in length) fused to form a cone at the centre of the corolla. In Europe, *S. dulcamara* is visited mostly by buzz-pollinating *Bombus spp.*, and, occasionally, hoverflies, which probe the base of the corolla and anther cone (Müller 1883, Macior 1964, Free 1970, Waser *et al.* 2011). Flowers of *S. dulcamara* were obtained from an experimental plant grown from seeds collected in Stirling, United Kingdom. The second species was *S. rostratum,* an annual species native to Mexico and the southern U.S.A., but which occurs outside its native range as a noxious weed. The flowers of *S. rostratum* are larger (34mm across), with four anthers presented loosely in the centre of the flower (7.5mm in length), and a fifth, enlarged (12.2mm), S-shaped anther located off the central axis of the flower (Vallejo-Marin *et al.* 2014). In the native range, *S. rostratum* is visited by a taxonomically and morphologically diverse range of bees, including bumblebees, honeybees and occasionally hoverflies (Linsley & Cazier 1963, Bowers 1975, Solis-Montero *et al.* 2015, Vega-Polanco *et al.* 2020). Experimental populations in Scotland are frequently visited by bumblebees (De Luca *et al.* 2014), and occasionally by hoverflies (MVM pers. obs.). The flowers of *S. rostratum* used in this experiment were obtained from two accessions from either the native (10s71, San Miguel de Allende, Mexico; 20.901°, -100.705°) or introduced ranges (10-TON-1, Tongzhou, China; 39.451°, 116.435°).

We induced a small number of hoverflies and bees to produce defence buzzes by holding one of their legs between two fingers, and gently pressing the insect against the anthers of buzz-pollinated *Solanum*. This experiment was conducted on 3-6 August using a subset of taxa available at

the time. For *S. dulcamara* we pressed the thorax of the insect against the fused anther cone. For *S. rostratum* we pressed the insect's thorax against the centrally located, four small anthers (feeding anthers). The insect was pressed against the anthers for about 3-5 seconds, in which it produced several defence buzzes. Pollen ejected from the anthers was caught in a small amount of fuchsine-glycerol gelatine placed on a microscope slide placed ~10mm from the anthers tips (Kearns & Inouye 1993). Similar manipulation of the anthers using a dead insect did not eject pollen. The presence of pollen grains on the slide was assessed using a compound microscope (CX31, Olympus, Southend-on-Sea, Essex). Due to the very large number of pollen grains expelled in most cases, we categorised each slide into one of three classes: no-pollen grains, <2,000 grains, and >2,000 grains.

Analysis of vibrations

We used a 20 Hz high-pass filter (Hanning window, window length=512 samples) on the recordings to remove low-frequency background noise using the *fir* function of the package *seewave* (Sueur *et al.* 2008) in *R* ver. 4.0.2 (R Core Development Team 2020). From each recording of each insect, we manually selected approximately 10 buzzes. This was carried out blind to the identity of the insect, avoiding introduction of any subconscious bias. A buzz was defined either as a discrete burst of vibration or, in cases where insects produced a continuous buzz for several seconds, we selected a section of it. Buzzes were chosen to capture events of full contact of the insect against the accelerometer, and to sample throughout the duration of the recording. We calculated peak acceleration amplitude (A_{PEAK}) for each buzz. We first obtained the amplitude envelope of each buzz using the *seewave* function *env,* and a smoothing function with a window size of 2 samples. We chose to study smoothed peak amplitude instead of other measurements of amplitude such as root mean squared acceleration (RMS) or non-smoothed peak acceleration because it captures the maximum accelerations produced by the insects, while removing potential artefactual spikes in the recording (Pritchard & Vallejo-Marín 2020b). However, preliminary analyses showed that, as expected, our chosen smoothed peak acceleration was strongly and positively correlated with RMS acceleration (Pearson's r=0.788, P<0.001) and with non-smoothed peak acceleration (r=0.971, P<0.001). We also estimated the fundamental frequency of each buzz using the function *fund* using a window length of 1,024 samples, an overlap of 50%, and a maximum frequency of 1,000Hz. The median fundamental frequency of the windowed analysis was calculated for each buzz and used for downstream analyses.

Statistical analyses

We used linear mixed effects models implemented in the package *lmer* (Bates *et al.* 2014) in *R* ver. 4.0.2 (R Development Core Team 2020). We used peak acceleration amplitude (A_{PEAK}) as the

response variable, sex, thorax width, order (Hymenoptera or Diptera) and their second and third order interactions as fixed effects, and species and individual as random effects. During model selection, we sequentially removed third order and second order interaction terms that were not statistically significant as assessed by a likelihood ratio test (LRT), while keeping all the main fixed effects. We analysed fundamental frequency of the thoracic vibrations (in Hz) using the same approach. The statistical significance of fixed effects was assessed using Type III sums of squares in the *R* package *lmerTest* (Kuznetsova *et al.* 2014). Model predictions for the fixed effects were plotted using the package *sjPlot* (Lüdeke 2018). A preliminary analysis using only taxa for which we had at least one male and one female individual per species (20 taxa), generated the same conclusions as the analysis of the entire data set (42 taxa), and we therefore report here only the results obtained with the whole data set.

Results

Diversity of insects sampled

We collected 318 insects in total representing 44 taxa: 113 individuals from 22 taxa of Diptera and 205 individuals from 22 taxa of Hymenoptera. Insects were identified to species in most cases, except for four bee taxa that were identified as morpho species to genus level only (*Andrena sp.* 1*, Andrena sp.* 2*, Colletes sp.* and *Lasioglossum sp.*). Individuals of the *Bombus terrestris/B. lucorum/B. cryptarum/B. magnus* species complex were treated as a single taxon (*B. terrestris-lucorum*) as identification of female workers in this complex using morphological characters is prone to error. A single individual of a hoverfly-mimic conopid fly, *Conops quadrifasciata* (Conopidae) was caught. For the remaining individuals, all Diptera belonged to the family Syrphidae, and Hymenoptera to the families Andrenidae (3 species), Apidae (17 species), Colletidae (1 species), Halictidae (1 species) and Megachillidae (2 species). On average we sampled 7.2 individuals per species (median 6, range 1-29). Individuals that did not buzz (number of individuals relative to the total collected for that species), were: 1/1 *Conops quadrifasciata,* 1/18 *Episyrphus balteatus,* 1/1 *Eristalis arbustorum,* 4/10 *Helophilus pendulus*, 1/6 *Rhingia campestris*, 1/8 *Andrena scotica*, 7/11 *Apis mellifera*, 1/13 *Bombus pascuorum*, 1/3 *Lasioglossum sp.*, and 1/2 *Osmia bicornis*. We were able to acquire thoracic vibrations for 299 individuals from 42 taxa (Table 1, Table S1). In total, we obtained vibrations from 94 male and 205 female individuals, including 11 bumblebee queens. Among bees, the best-represented taxonomic group was bumblebees (*Bombus spp.*) with 166 individuals from 13 taxa, including two cuckoo bumblebees, *B. bohemicus* and *B. sylvestris*, and two geographically restricted forms collected from the Outer Hebrides, which were treated as separate taxa (*B. muscorum agricolae* and *B. jonellus hebridensis*). Thorax size for 2/16 individuals of *B. hypnorum* was not

collected and the specimens lost. At the genus level, all the bees analysed here are reported to buzz-pollinate, with the exception of the honeybee, *Apis mellifera* (Cardinal *et al.* 2018). In contrast, none of the studied fly species has been reported to buzz-pollinate.

Insect thoracic vibrations

Figure 3 shows an example of recorded vibrations for two species of bees (*Bombus muscorum* and *Megachile willughbiella*) and two species of hoverflies (*Volucella bombylans* and *Episyrphus balteatus*) of contrasting thorax size. We analysed 3,918 non-flight thoracic vibrations (defence buzzes) in total, with 13.10 defence buzzes on average per individual (median=10 buzzes, range 1–50). Defence buzzes produced by bees and flies ranged widely in both amplitude (mean V_{PEAK}=123.7ms^{-2}, range=2–588ms^{-2}) and fundamental frequency (mean=206Hz, range=28–465) (Table 1; Figure 4). Although in many cases the fundamental frequency also corresponded to the dominant frequency (Figure 3F-H), some recordings showed dominant frequencies at higher harmonics (Figure 3E).

For peak acceleration (A_{PEAK}), we did not detect significant third or second order interactions among the fixed effects (sex, size and insect Order) (*p*-values > 0.05 as assessed by LRT). In the final model, we observed a significant positive effect of thorax size on A_{PEAK} (coefficient=42.415, *p*-value<0.001; Figure 5, Table 2) but a negative effect of sex, with males producing on average lower amplitude vibrations than females (coefficient=-16.406, *p*-value=0.019). In contrast, both bees and hoverflies produced similar acceleration amplitudes after accounting for sex and thorax size (coefficient for insect Order=-12.892, *p*-value=0.165; Figure 5). In the case of fundamental frequency, we detected a significant interaction between thorax size and insect Order (LRT *p*-value<0.001) and thus the final selected model preserved this second-order term. Analysis of this model showed that the fundamental frequency of non-flight vibrations differed significantly between bees and hoverflies with the latter producing on average higher-frequency vibrations (coefficient=-190.604, *p*-value<0.001). The effect of thorax size on fundamental frequency differed between bees and hoverflies (Table 2). For hoverflies, increased thorax size was associated with lower frequency vibrations, while in bees, thorax size showed a shallow, positive association with vibration frequency (Figure 5). In contrast to the pattern observed for A_{PEAK}, there was no effect of sex on the fundamental frequency of thoracic vibrations (coefficient for males=-0.969, *p*-value=0.909).

Pollen release

For this experiment we were able to qualitatively assess pollen release in 48 flowers of buzz-pollinated *Solanum dulcamara* (n=16 flowers) and *S. rostratum* (n=17 and n=15, for accessions 12-TON-1 and 10-s-71, respectively). We analysed 12 hoverflies from four species: *Platycherius albimanus* (n=1 individual), *Syrphus vitripennis* (n=3), *S. ribesii* (n=2), *Eristalis pertinax* (n=5), and *Volucella pellucens* (n=1). Thorax size of the hoverflies tested varied from 1.80 mm (*P. albimanus*) to 5.24 mm (*V. pellucens*). We also obtained pollen release samples from 5 bees in four taxa: *Lasioglossum sp.* (n=1), *Colletes sp.* (n =2), *Bombus lapidarius* (n=1, male), and *B. sylvestris* (n=1, male). Thorax size in these bees varied from 2.59 mm (*Lasioglossum sp.*) to 5.14 mm (*Bombus sylvestris*). In the majority of cases (39/48 flowers), defence buzzes by both flies and bees resulted in significant amounts of pollen ejected (well in excess of 2,000 grains). In 2/48 flowers no pollen was released (*S. vitripennis* on *S. rostratum*, and *B. sylvestris* on *S. dulcamara*), and in 7/48 cases <2,000 pollen grains were ejected (flies on both *S. dulcamara* and *S. rostratum*).

Discussion

Buzz-pollinating bees

Our study shows that even within bees there is a wide range of variation in the non-flight thoracic vibrations produced. Although variation in buzz frequency among bee species is known from acoustic studies of buzz pollination (Burkart *et al.* 2011, De Luca *et al.* 2019, Rosi-Denadai *et al.* 2020), much less is known about variation in vibration amplitude. The lowest peak amplitude among bees was produced by *Apis mellifera* (Table 1, Figure 6), consistent with the study of King & Buchmann (2003) that determined that honeybee vibrations cannot reach the acceleration required to remove pollen from the poricidal flowers of *Solanum* and kiwifruit (*Actinidia deliciosa*). Similarly, *Megachille willoughbiela* and *O. bicornis* (Megachilidae) produced relatively low acceleration amplitudes but above those expected to remove pollen from flowers (King & Buchmann 2003). Regarding these two species, *M. willoughbiella* has been observed buzz-pollinating tomato flowers (Teppner 2005), while some species of *Osmia* but not *O. bicornis,* can buzz pollinate (Cane 2014). All remaining bee species in this study produce thoracic vibrations with acceleration amplitudes well above those required to remove pollen from buzz-pollinated flowers (King & Buchmann 2003, Kemp & Vallejo-Marin 2020).

Pollen removal from buzz-pollinated flowers

Vibrations are not the only way to extract pollen from poricidal anthers (Buchmann 1983). For example, *Megachile addenda* rubs the poricidal anthers of cranberry (*Vaccinium* subg. *Oxycoccus,* Ericaceae) with the forelegs to release pollen, while *Osmia ribifloris* and *Apis mellifera* tap anthers of cranberry or blueberry (*Vaccinium* sect. *Cyanococcus*) (Thorp 2000). Some bees (e.g., *A. mellifera*) and hoverflies probe the anther pore with their proboscis or gather pollen previously ejected by buzz-pollinators (Müller 1883, Solis-Montero *et al.* 2015, Vega-Polanco *et al.* 2020). Well-known non-buzzing pollen thieves, e.g., *Trigona spp.*, can also chew through the anther wall to access pollen (Renner 1983). In Europe, relatively common hoverflies including *Eristalis tenax, Syritta pipiens*, *Sphaeorphoria sp.*, and *Melanostoma mellinum* collect pollen from flowers of *Solanum tuberosum* and *S. nigrum*, and *Rhingia rostrata* visits flowers of *Solanum dulcamara* probing the anther pores with its mouthparts (Müller 1883). However, vibrating anthers remains unrivalled as an extremely rapid and effective mechanism for removing large amounts of pollen (Harder & Barclay 1994, Vallejo-Marin *et al.* 2009, Kemp & Vallejo-Marin 2020).

Why is buzz pollination so rare in hoverflies?

If hoverflies can produce buzzes of similar acceleration amplitude as buzz pollinating bees, and as we have shown, defence buzzes by hoverflies as small as 1.80mm in thorax width can remove pollen from buzz-pollinated flowers, why don't more hoverflies use vibrations on flowers? There are a number of non-mutually exclusive hypotheses that can explain the lack of buzz-pollinating hoverflies (summarised in Table 3). These hypotheses can be broadly classified into mechanical, behavioural and life history explanations. We have shown that most of the studied bees and hoverflies are capable of producing vibrations of sufficient amplitude to remove pollen, and that the peak acceleration of these vibrations increases with insect size, providing little support for the amplitude limitation mechanical hypothesis. Other mechanical explanations include the hypothesis that flies lack the morphology required to firmly grasp the flower while producing non-flight thoracic vibrations. Bees use their mandibles to hold on to the anthers or other floral tissues while vigorously shaking. The vibrations are then transmitted to the flower by direct contact of the thorax, abdomen, head and to a much lesser extent, the legs (King & Buchmann 2003). It is possible that a weak attachment to the flower prevents mandible-less flies from holding on to the flowers firmly enough to allow efficient transmission of the vibrations from the thorax to the anthers. Although the fly can hold on to the anthers with its legs, the jointed nature of arthropod legs may make them much poorer channels to transmit vibrations to the substrate (King & Buchmann 2003, Cocroft & Rodríguez 2005).

359 Behavioural explanations include the hypothesis that hoverflies are incapable of repurposing
360 a behaviour (thoracic vibrations) from one context (e.g. defence) to another (pollen removal). We
361 consider this hypothesis unlikely, given the behavioural flexibility of flies in other contexts (Penney
362 *et al.* 2014), although there is no direct experimental evidence showing that flies can learn to buzz
363 pollinate. Additionally, lack of buzz-pollinating hoverflies may be explained through an optimal
364 foraging/energetics hypothesis. Producing thoracic vibrations is an energetically costly behaviour
365 (Casey *et al.* 1985, King *et al.* 1996), compared to, for example, probing the anther pores with the
366 mouth parts. Even within bumblebees, pollen collection without buzzing seems to be preferred
367 when pollen can be efficiently extracted via other means, which suggests that buzzing is costly
368 (Switzer *et al.* 2019). If the energetic investment required to produce floral vibrations offsets the
369 fitness returns of releasing large amounts of pollen from a single flower, then floral vibrations will
370 not represent an optimal foraging strategy (Harder 1990). Finally, it could be hypothesised that
371 producing floral vibrations also accrues non-energetic costs. A by-product of floral vibrations is the
372 characteristic high-pitched noise that gives the name to buzz pollination. This sound can be heard at
373 a distance and is possible that buzzing is disadvantageous if it gives away the position of floral
374 visitors to potential predators. Yet, given that buzzing is an alarm or aposematic signal in bees and
375 many hoverflies are Batesian mimics of bees and wasps (Rashed *et al.* 2009, Moore & Hassall 2016),
376 selection for silent floral visitors appears unlikely.

377 Life history explanations provide a compelling class of hypotheses for the lack of buzz-
378 pollinating hoverflies. Differences in the pollen requirements between bees and flies during larval
379 development might select for very different strategies to remove pollen from flowers. Hoverfly
380 larvae rely mostly on non-pollen nutrition for their development, parasitising other insects,
381 inhabiting environments that are rich in other organic matter, or in some cases parasitising the nests
382 of other bees including social bees and consuming bee-collected resources (Schmid-Hempel 1995,
383 McAlister 2017). In contrast bees collect large amounts of pollen to transport back to their nest and
384 provide food for developing larvae (Müller *et al.* 2006). The higher pollen requirement in bees favour
385 strategies that allow them to rapidly collect large amounts of pollen from flowers such as producing
386 floral vibrations, while hoverflies are not under similar selective pressures (provisioning of young
387 hypothesis). This hypothesis also predicts that bees that do not regularly collect large amounts of
388 pollen, including social parasites (e.g., cuckoo bumblebees), male bees, and other exceptional bees
389 should not deploy vibrations to remove pollen from flowers despite possessing the potential to
390 produce vibrations of sufficient amplitude. Our study shows that species of obligate social parasites
391 (cuckoo bumblebees *B. sylvestris* and *B. bohemicus*), which lack corbiculae and rely on the host
392 workers to collect pollen and nectar for larval feeding (Lhomme & Hines 2019), produced vibrations

with similar high acceleration amplitude to those used by buzz-pollinating bumblebees on flowers (Arroyo-Correa *et al.* 2019). Similarly, although male bees reached lower acceleration than females, both sexes of most bee species are able to generate vibrations capable of removing pollen from buzz-pollinated flowers (King & Buchmann 2003, Kemp & Vallejo-Marin 2020).

Our study has shown that hoverflies are capable of producing defence vibrations with similar acceleration amplitude to buzz-pollinating bees, and that these vibrations are sufficient to remove pollen from different species of buzz-pollinated flowers. We believe that differences in pollen requirements between bees and hoverflies provide a compelling hypothesis for the dearth of buzz-pollinating flies. However, additional work is required to explicitly address this and other explanations for the difference in buzz-pollination capacity among flies and bees. Moreover, the question of why some bees, but not others, buzz-pollinate remains unanswered. Further comparisons of buzz-pollinating and non-buzz-pollinating bees with different morphologies, behaviours, and life histories could help addressing this question.

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573 **Table 1.** Summary statistics of the characteristics of non-flight thoracic vibrations (defence buzzes) and thorax size from 299 individuals in 42 taxa of

574 hoverflies (Syrphidae) and bees (Hymenoptera: Apoidea: Anthophila). Number of buzzes analysed: N=3,918 buzzes. Mean ± SE.

Species	Peak amplitude ($m\ s^{-2}$)	Fundamental Frequency (Hz)	N buzzes	Thorax width (mm)	N bees
Diptera					
Cheilosia illustrata	152.89 ± 15.37	297.2 ± 29.87	100	3.86 ± 1.29	10
Epistrophe grossulariae	44.09 ± 5.23	225.17 ± 26.72	72	3.52 ± 2.49	3
Episyrphus balteatus	59.05 ± 4.16	320.89 ± 22.63	202	2.42 ± 0.61	17
Eristalis horticola	144.96 ± 27.4	190.84 ± 36.06	29	3.69 ± 2.61	3
Eristalis interruptus	117.86 ± 25.72	210.91 ± 46.03	22	4.14 ± 4.14	2
Eristalis intricarius	71.23 ± 23.74	178.19 ± 59.4	10	4.23	1
Eristalis pertinax	153.95 ± 15.17	211.09 ± 20.8	104	3.96 ± 1.32	10
Ferdinandia cuprea	35.78 ± 8.21	233.58 ± 53.59	20	3.16	1
Helophilus pendulus	79.83 ± 8.19	225.93 ± 23.18	96	3.65 ± 1.63	6
Leucozona lucorum	149.39 ± 49.8	223.62 ± 74.54	10	3.50	1
Merodon equestris	139.27 ± 16.77	196.94 ± 23.71	70	4.83 ± 2.41	5
Myathropa florea	62 ± 11.72	140.8 ± 26.61	29	3.96	1
Platycheirus albimanus	51.06 ± 5.6	275.1 ± 30.2	84	1.64 ± 0.73	6
Rhingia campestris	96.62 ± 14.57	249.29 ± 37.58	45	3.15 ± 1.57	5
Scaeva pyrastris	97.84 ± 22.45	281.94 ± 64.68	20	3.66 ± 3.66	2
Sericomyia silentis	151.22 ± 17.12	177.51 ± 20.1	79	4.57 ± 2.28	5
Syrphus ribesii	59 ± 5.43	300.64 ± 27.68	119	2.75 ± 0.92	10
Syrphus vitripennis	70.17 ± 9.14	210.05 ± 27.35	60	2.85 ± 1.28	6
Volucella bombylans	144.76 ± 13.27	186.33 ± 17.08	120	5.14 ± 1.94	8
Volucella pellucens	176.64 ± 32.8	140.7 ± 26.13	30	4.79 ± 3.39	3
Hymenoptera					

Andrena scotica	128.98 ± 12.71	150.65 ± 14.84	104	3.60 ± 1.47	7
Andrena sp. 1	96.99 ± 18.01	138.13 ± 25.65	30	3.40 ± 3.4	2
Andrena sp. 2	99.4 ± 14.06	163.25 ± 23.09	51	2.99 ± 1.72	4
Anthophora furcata	172.33 ± 57.44	127.8 ± 42.6	10	4.37	1
Apis mellifera	32.55 ± 4.91	119.26 ± 17.98	45	3.56 ± 2.05	4
Bombus bohemicus	153.91 ± 16.22	199.83 ± 21.06	91	5.27 ± 2.15	7
Bombus hortorum	142.7 ± 9.21	189.46 ± 12.23	241	4.83 ± 1.17	18
Bombus hypnorum	141.41 ± 10.02	222.49 ± 15.77	200	4.76 ± 1.23	16
Bombus jonellus	127.42 ± 13.99	167.58 ± 18.39	84	4.27 ± 1.74	7
Bombus jonellus hebridensis	129.32 ± 9.83	180.3 ± 13.71	174	4.05 ± 1.17	13
Bombus lapidarius	125.35 ± 8.76	207.37 ± 14.48	206	4.57 ± 1.14	17
Bombus monticola	125.24 ± 41.75	191.02 ± 63.67	10	3.95	1
Bombus muscorum	201.98 ± 15.72	159.65 ± 12.43	166	5.04 ± 1.35	15
Bombus muscorum agricolae	193.43 ± 14.75	220.64 ± 16.82	173	4.73 ± 1.67	9
Bombus pascuorum	139.53 ± 11.92	201.54 ± 17.22	138	4.54 ± 1.37	12
Bombus pratorum	101.48 ± 7.85	194.28 ± 15.03	168	3.88 ± 1.23	11
Bombus sylvestris	140.11 ± 10.68	175.64 ± 13.39	173	5.01 ± 1.58	11
Bombus terrestris-lucorum	144.17 ± 7.46	182.43 ± 9.45	374	5.09 ± 0.96	29
Colletes sp.	67.36 ± 12.73	140.11 ± 26.48	29	3.06 ± 3.06	2
Lasioglossum sp.	62.13 ± 12.96	225.7 ± 47.06	24	2.07 ± 2.07	2
Megachile willughbiella	63.31 ± 7.85	242.72 ± 30.11	66	4.30 ± 2.15	5
Osmia bicornis	57.38 ± 9.19	117.31 ± 18.78	40	4.01	1

575

576 **Table 2.** Analysis of peak acceleration (**A**) and fundamental frequency (**B**) of non-flight thoracic
577 vibrations (defence buzzes). Separate linear mixed-effects models were fitted for each response
578 variable (amplitude or frequency) using species and individual as random effects and insect Order,
579 thorax size and sex as fixed effects. The table shows statistical tests of significance for fixed effects
580 calculated using Type III sums of squares with Satterthwaite's correction. Sample sizes in both
581 models are: 3,884 observations, 296 individuals, and 42 taxa.

582 **A. Peak acceleration amplitude (ms^{-2})**

	Estimate	Std. Error	*p*-value
Intercept	-37.572	15.679	
Order (Hymenoptera)	-12.892	9.098	0.165
Thorax size (mm)	42.415	4.076	<0.001
Sex (male)	-16.406	6.948	0.019

583

584 **B. Fundamental frequency (Hz)**

	Estimate	Std. Error	*p*-value
Intercept	351.886	31.341	
Order (Hymenoptera)	-190.604	42.773	<0.001
Thorax size (mm)	-32.857	8.417	0.011
Sex (male)	-0.970	8.509	0.909
Order * size	37.960	10.569	<.001

585

586

587 **Table 3.** Hypotheses explaining why most flies do not use vibrations to remove pollen from flowers
588 with poricidal anthers (buzz-pollinated flowers).

Type	Hypothesis	Brief description
Mechanical-Morphological	Amplitude limitation	Hoverflies cannot produce vibrations of sufficient amplitude to release pollen from poricidal anthers.
	Weak attachment	Hoverflies, in part because they lack mandibles, cannot hold onto the flower to transmit thoracic vibrations to the flower effectively.
Behavioural	Behavioural inflexibility	Inability to repurpose a behaviour (thoracic vibrations) from one context (defence) to another (pollen removal).
	Energetics/Optimal foraging	The energy required to produce floral vibrations is more costly than the benefit from accessing the pollen through vibrations; sufficient pollen can be removed without producing costly vibrations.
	Predator attraction	The sound of floral vibrations, which can be heard from a few meters away, may attract potential predators.
Life History	Provisioning of young	In flies, pollen is consumed by the adults during floral visitation and used by females before egg laying. In contrast, bees also have to collect and transport pollen back to their nests to provide developing larvae. The higher pollen requirement in bees favour strategies that allow them to rapidly collect and transport large amounts of pollen, while the same does not apply in hoverflies.
	Sociality	This could be an extension of the *Provisioning of young* hypothesis. In social species, pollen provision might exceed the requirements of a solitary bee, particularly favouring rapid collection of large amounts of pollen.

589

590 **Figure 1.** Hoverflies and bees use different strategies to collect pollen from buzz-pollinated flowers.
591 (A) Marmalade hoverfly (*Episyrphus balteatus*, Syrphidae) gathers pollen from the anther pores
592 using its mouth parts in a flower of buffalo bur (*Solanum rostratum*, Solanaceae) in Scotland. (B)
593 Buff-tailed bumblebee (*Bombus terrestris audax*) uses vibrations from its thoracic muscles to rapidly
594 remove large amounts of pollen in an experimental flower of *S. rostratum*. Photo credits: (A) Phil
595 Friston-Reilly; (B) Mario Vallejo-Marín.

596 **Figure 2.** Experimental set up to acquire non-flight thoracic vibrations of bees and flies. Briefly, a
597 chilled insect was tethered between the head and the thorax using a loop made of nylon thread held
598 at the tip of a metal syringe needle with a blunted end. The insect's thorax was gently but firmly
599 pressed against a miniature piezoelectric accelerometer (PCB 352C23) as shown in the diagram. The
600 accelerometer was attached to the end of a split bamboo flower stick by 30mm of connecting
601 electrical cable. The cable was attached to the stick with tape. The stick was held in place by
602 attaching it to a plastic container through one hole in each end. The voltage signal generated by the
603 accelerometer was acquired using a CompactDAQ system consisting of a NI chassis (NI cDAQ-9171)
604 and a C-Series input module with 24-bit resolution (NI 9250). The data was recorded on a PC laptop
605 using custom software written in LabView. Full description of the experimental set up is provided in
606 the Materials and Methods section.

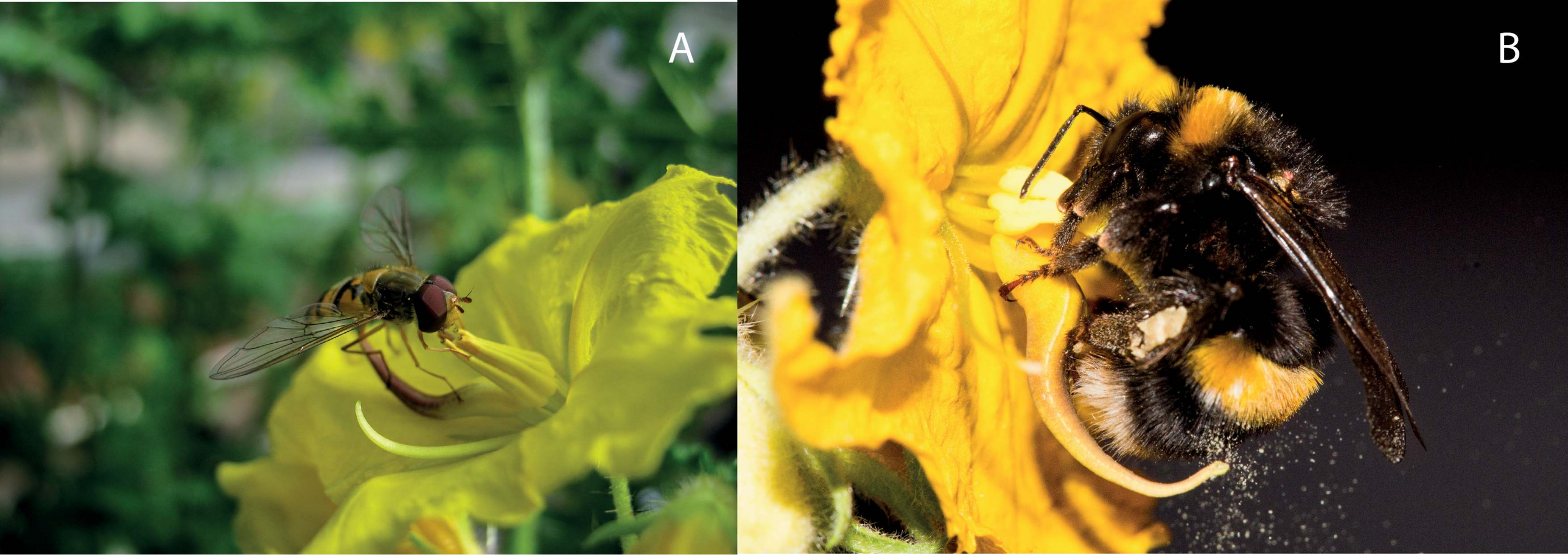
607 **Figure 3.** Examples of non-flight thoracic vibrations ("defence buzzes") of two species of hoverflies
608 (Diptera: Syrphidae): the large parasitic bumblebee mimic hoverfly, *Volucella bombylans* (panels A,E)
609 and the small marmalade hoverfly, *Episyrphus balteatus* (D, H); and two bee species (Hymenoptera:
610 Apidae and Megachillidae): the moss carder bumblebee, *Bombus muscorum* (B, F), and the
611 Willoughby's leafcutter bee, *Megachile willoughbiella* (D, H). Panels A-D show two recorded seconds
612 of defence vibrations in the time domain. A single buzz, or buzz section, selected for subsequent
613 analysis is shown in teal colour. The dotted horizontal line shows the peak amplitude acceleration of
614 the selected buzz. Panels E-H show the selected buzz in the frequency domain, specifically the power
615 spectrum density (PSD). The dashed vertical lines show the harmonic series (<1kHz) of the
616 fundamental frequency (the first peak in the PSD).

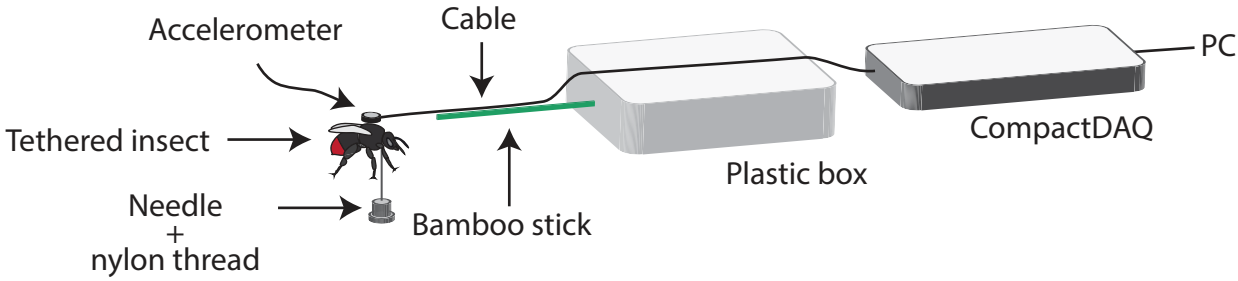
617 **Figure 4.** Box-plots showing the within- and among-species variation in peak acceleration amplitude
618 (top panel) and fundamental frequency (bottom panel) of non-flight thoracic vibrations (defence
619 buzzes) in bees (Hymenoptera: Andrenidae, Apidae, Colletidae, Halictidae and Megachillidae) and
620 hoverflies (Diptera: Syrphidae).

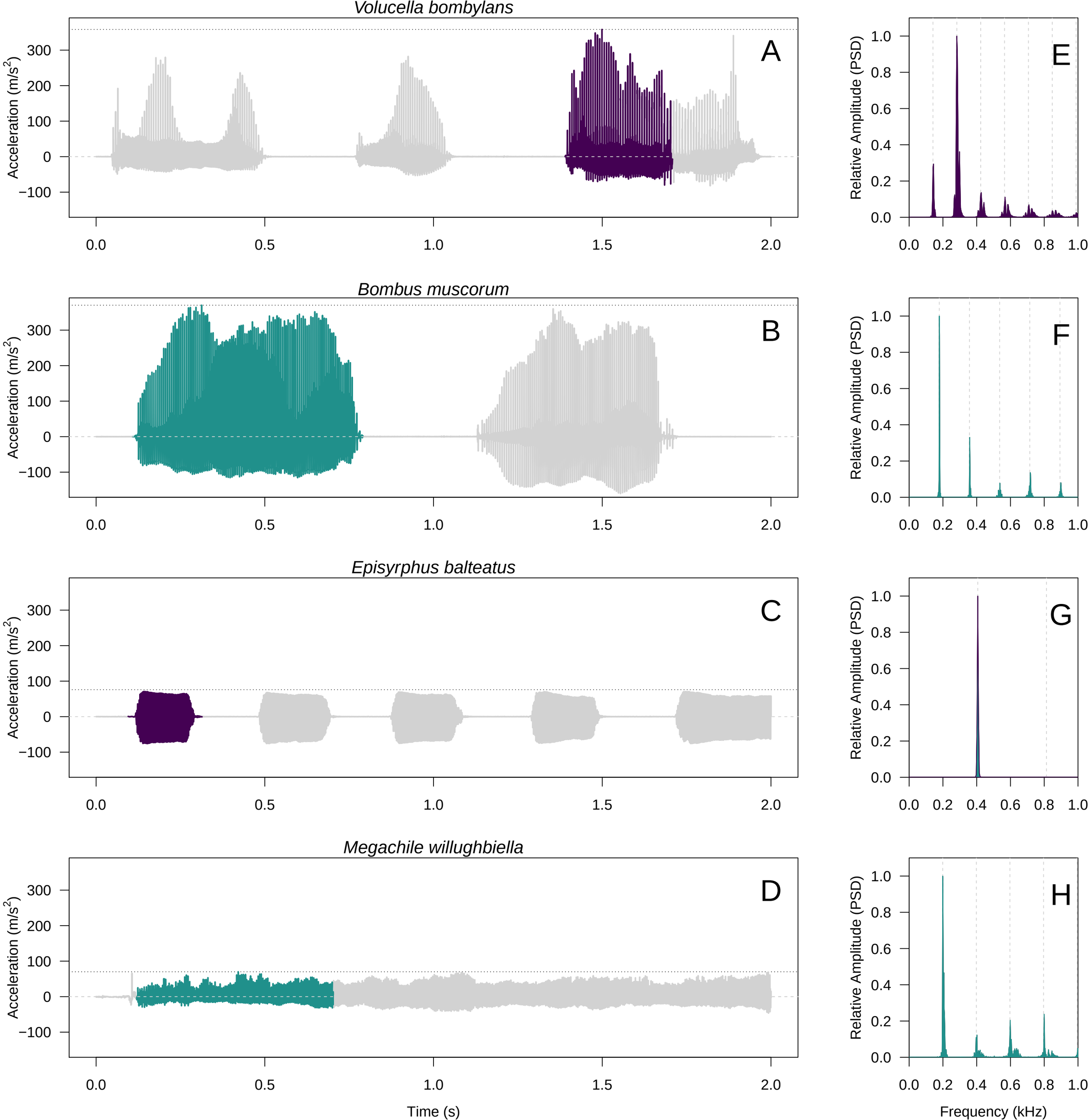
621 **Figure 5.** Relationship between thorax width and (A) peak acceleration amplitude or (B) fundamental
622 frequency of non-flight thoracic vibrations (defence vibrations) of hoverflies (Diptera, purple) and

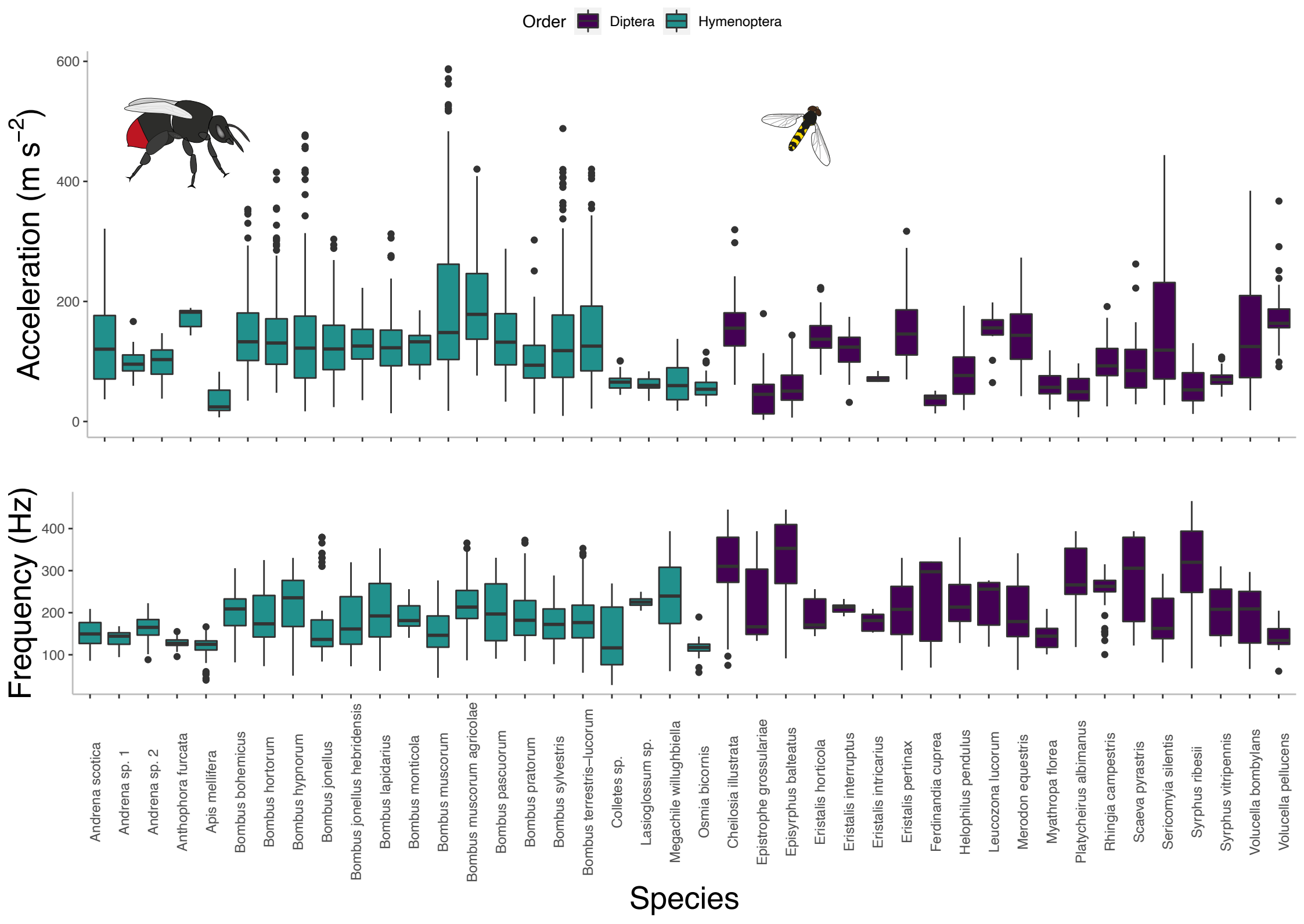
bees (Hymenoptera, green). Observed values are shown with closed symbols (n=3,884 buzzes, 296 insects, 42 taxa). The lines represent the predicted values (marginal effects) of the response variable (amplitude or frequency) for a range of thorax width values, estimated with linear mixed-effects models. The shaded area corresponds to the 95% confidence intervals of the predicted values.

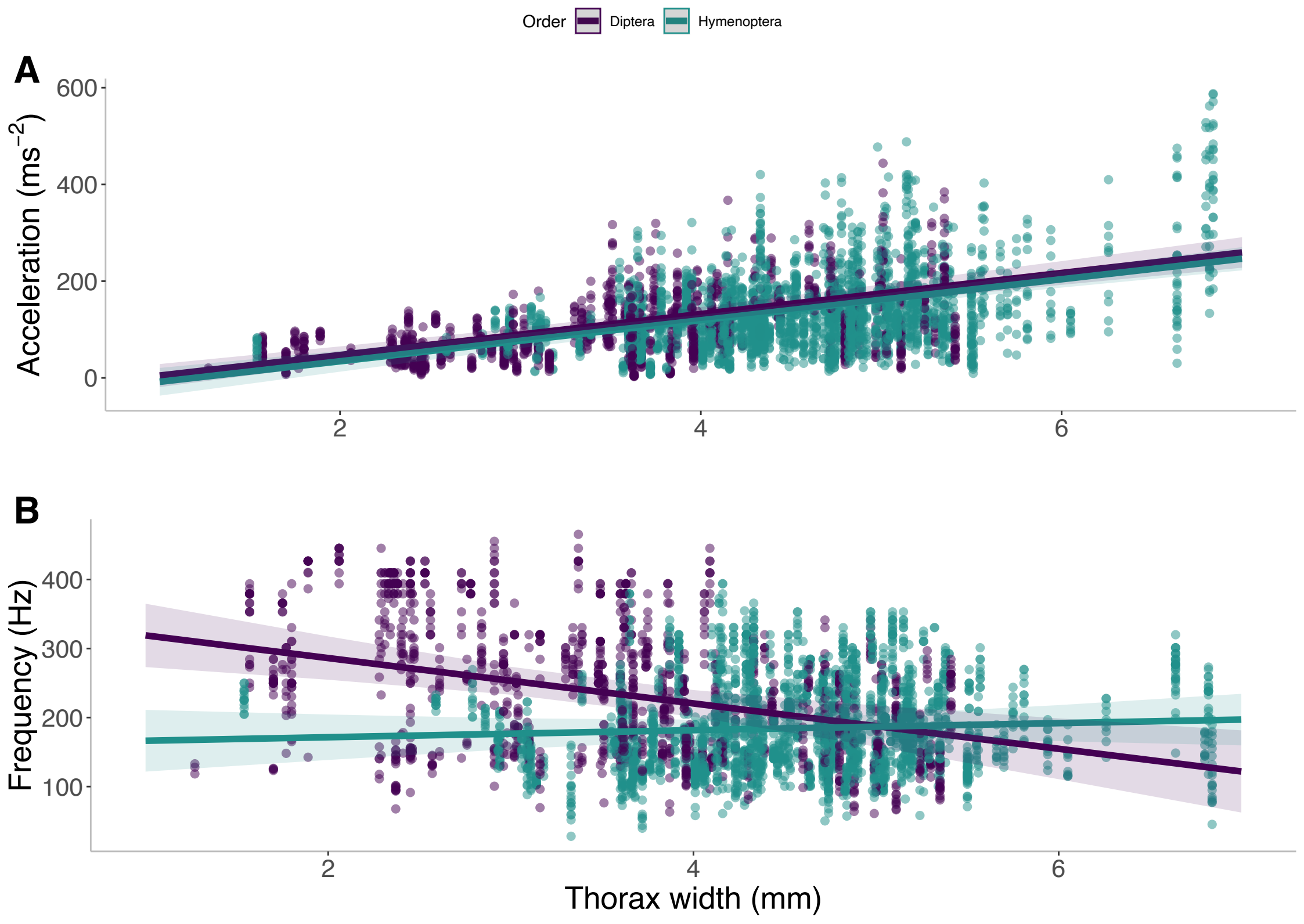
Figure 6. Relationship between peak acceleration amplitude and thorax width for 42 taxa of hoverflies (Diptera) and bees (Hymenoptera). All the bee genera shown here are known to buzz-pollinate, with the exception of *Apis mellifera* (Am), while none of the hoverfly genera shown here have been observed buzz-pollinating. The only published observation of a buzz-pollinating fly is for *Copestylum mexicanum* (formerly *Volucella mexicana*). Only the mean values are shown for each species. The size of the symbols is proportional to the number of buzzes analysed. Species key: As=*Andrena scotica,* Asp1=*Andrena sp. 1,* Asp2=*Andrena sp. 2, Af=Anthophora furcata,* Am=*Apis mellifera,* Bb=*Bombus bohemicus,* Bho=*Bombus hortorum,* Bhy=*Bombus hypnorum,* Bj=*Bombus jonellus,* Bjh=*Bombus jonellus hebridensis,* Bl=*Bombus lapidarius,* Bmo=*Bombus monticola,* Bm=*Bombus muscorum,* Bma=*Bombus muscorum agricolae,* Bpa=*Bombus pascuorum,* Bpr=*Bombus pratorum,* Bs=*Bombus sylvestris,* Btl=*Bombus terrestris-lucorum,* Csp=*Colletes sp.,* Lsp=*Lasioglossum sp.,* Mw=*Megachile willughbiella,* Ob=*Osmia bicornis,* Ci=*Cheilosia illustrata,* Eg=*Epistrophe grossulariae,* Eb=*Episyrphus balteatus,* Eh=*Eristalis horticola,* Eip=*Eristalis interruptus,* Eic=*Eristalis intricarius,* Ep=*Eristalis pertinax,* Fc=*Ferdinandia cuprea,* Hp=*Helophilus pendulus,* Ll=*Leucozona lucorum,* Me=*Merodon equestris,* Mf=*Myathropa florea,* Pa=*Platycheirus albimanus,* Rc=*Rhingia campestris,* Sp=*Scaeva pyrastris,* Ss=*Sericomyia silentis,* Sr=*Syrphus ribesii,* Sv=*Syrphus vitripennis,* Vb=*Volucella bombylans,* Vp=*Volucella pellucens*.

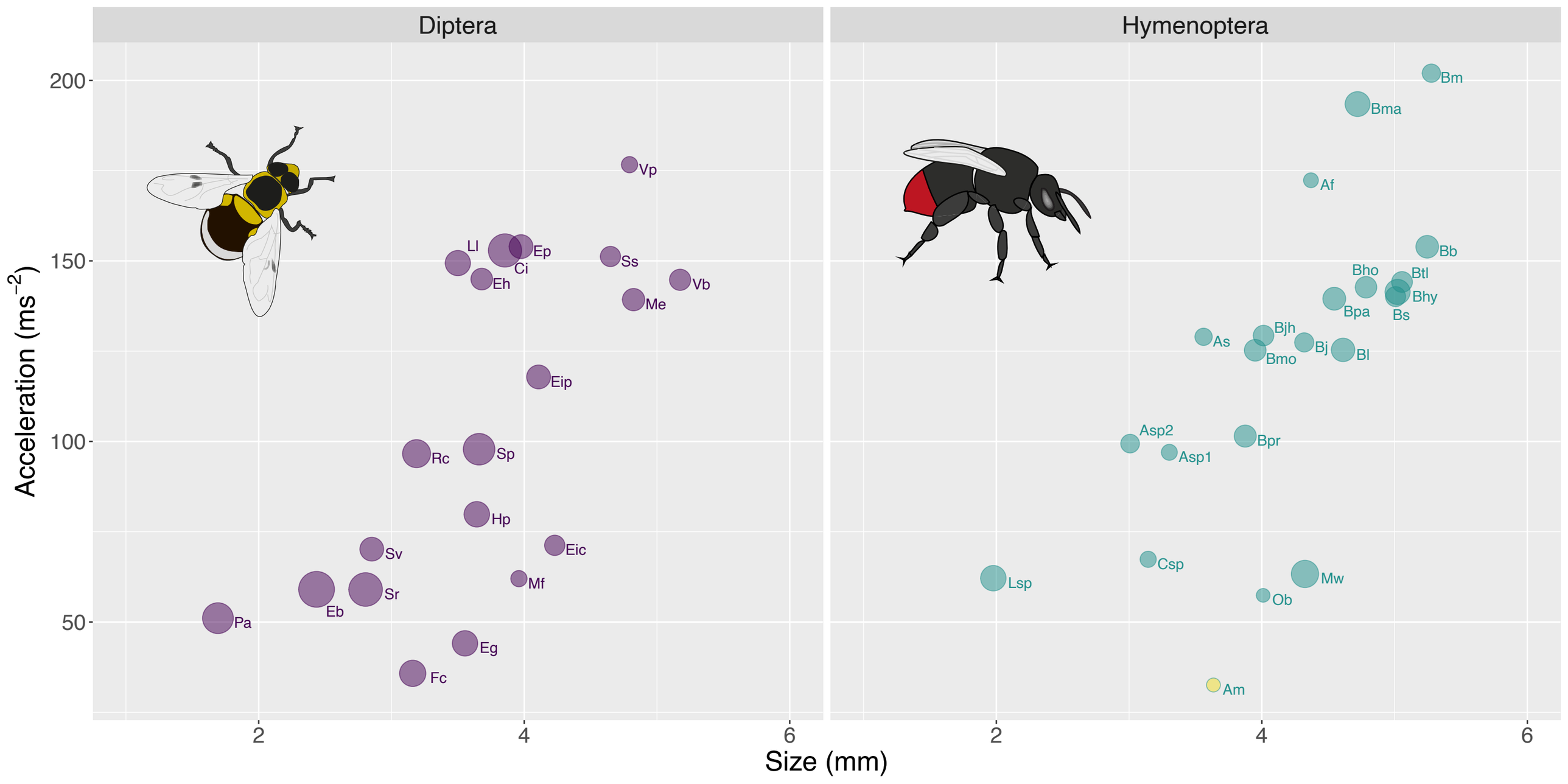

A
B


Accelerometer
Cable
PC
Tethered insect
CompactDAQ
Plastic box
Needle
+
nylon thread
Bamboo stick


Volucella bombylans
Bombus muscorum
Episyrphus balteatus
Megachile willughbiella
A
B
C
D
E
F
G
H
Acceleration (m/s2)
Relative Amplitude (PSD)
Time (s)
Frequency (kHz)


Order
Diptera
Hymenoptera
Acceleration (m s^{-2})
600
400
200
0
Frequency (Hz)
400
300
200
100
Andrena scotica
Andrena sp. 1
Andrena sp. 2
Anthophora furcata
Apis mellifera
Bombus bohemicus
Bombus hortorum
Bombus hypnorum
Bombus jonellus
Bombus jonellus hebridensis
Bombus lapidarius
Bombus monticola
Bombus muscorum
Bombus muscorum agricolae
Bombus pascuorum
Bombus pratorum
Bombus sylvestris
Bombus terrestris–lucorum
Colletes sp.
Lasioglossum sp.
Megachile willughbiella
Osmia bicornis
Cheilosia illustrata
Epistrophe grossulariae
Episyrphus balteatus
Eristalis horticola
Eristalis interruptus
Eristalis intricarius
Eristalis pertinax
Ferdinandia cuprea
Helophilus pendulus
Leucozona lucorum
Merodon equestris
Myathropa florea
Platycheirus albimanus
Rhingia campestris
Scaeva pyrastris
Sericomyia silentis
Syrphus ribesii
Syrphus vitripennis
Volucella bombylans
Volucella pellucens
Species


Order
Diptera
Hymenoptera
A
Acceleration (ms^{-2})
600
400
200
0
2
4
6
B
Frequency (Hz)
400
300
200
100
2
4
6
Thorax width (mm)


Diptera
Hymenoptera
Acceleration (ms^{-2})
Size (mm)
200
150
100
50
2
4
6
Vp
Ll
Ep
Ci
Eh
Ss
Vb
Me
Eip
Rc
Sp
Hp
Sv
Eic
Mf
Eb
Sr
Pa
Eg
Fc
Bm
Bma
Af
Bb
Bho
Btl
Bhy
Bpa
Bs
Bjh
As
Bj
Bmo
Bl
Asp2
Bpr
Asp1
Csp
Lsp
Mw
Ob
Am

1 **Table S1.** Number of individuals per species with recorded non-flight thoracic vibrations (defence buzzes). For social bees, female workers and queens are

2 shown separately.

Order	Family	Species	Male	Female	Queen	Total individuals	Number of buzzes
Diptera	Syrphidae	*Cheilosia illustrata*	6	4	--	10	100
		Epistrophe grossulariae	1	2	--	3	72
		Episyrphus balteatus	4	13	--	17	202
		Eristalis horticola	1	2	--	3	29
		Eristalis interruptus	1	1	--	2	22
		Eristalis intricarius	1	0	--	1	10
		Eristalis pertinax	5	5	--	10	104
		Ferdinandia cuprea	0	1	--	1	20
		Helophilus pendulus	5	1	--	6	96
		Leucozona lucorum	0	1	--	1	10
		Merodon equestris	2	3	--	5	70
		Myathropa florea	0	1	--	1	29
		Platycheirus albimanus	1	5	--	6	84
		Rhingia campestris	3	2	--	5	45
		Scaeva pyrastris	0	2	--	2	20
		Sericomyia silentis	5	0	--	5	79
		Syrphus ribesii	4	6	--	10	119
		Syrphus vitripennis	1	5	--	6	60
		Volucella bombylans	1	7	--	8	120
		Volucella pellucens	2	1	--	3	30
Diptera			**43**	**62**	**--**	**105**	**1321**
Hymenoptera	Andrenidae	*Andrena scotica*	0	7	--	7	104

		Andrena sp. 1	0	2	--	2	30
		Andrena sp. 2	0	4	--	4	51
	Apidae	*Anthophora furcata*	0	1	--	1	10
		Apis mellifera	0	4	0	4	45
		Bombus bohemicus	7	0	0	7	91
		Bombus hortorum	3	14	1	18	241
		Bombus hypnorum	7	8	1	16	200
		Bombus jonellus	1	4	2	7	84
		Bombus jonellus hebridensis	0	13	0	13	174
		Bombus lapidarius	4	13	0	17	206
		Bombus monticola	0	1	0	1	10
		Bombus muscorum	0	10	5	15	166
		Bombus muscorum agricolae	0	9	0	9	173
		Bombus pascuorum	0	11	1	12	138
		Bombus pratorum	4	7	0	11	168
		Bombus sylvestris	11	0	0	11	173
		Bombus terrestris-lucorum	13	15	1	29	374
	Colletidae	*Colletes sp.*	1	1	--	2	29
	Halictidae	*Lasioglossum sp.*	0	2	--	2	24
	Megachilidae	*Megachile willughbiella*	0	5	--	5	66
		Osmia bicornis	0	1	--	1	40
Hymenoptera			**51**	**132**	**11**	**194**	**2597**
Total			**94**	**194**	**11**	**299**	**3918**

3

4