PROPENSITY TO EFFICIENTLY TRANSMIT VIBRATIONS IN SNAPDRAGONS IN RESPONSE TO VIBROACOUSTIC SIGNALLING

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The coevolution of angiosperms and pollinating insects has drawn a diverse repertoire of plant-pollinator interactions leading to different mechanisms of pollen transfer. *Buzz pollination* is a dynamic pollen removal process between plants and insects that is thought to have emerged due to ecological and evolutionary factors. The floral morphology in buzz-pollinated flowers restricts the pollen access to non-pollinating insects to increase the conditions that favour fertilisation. Efficient pollinators such as bumblebees use sonication to vibrate the anthers of the flower, thereby causing pollen grains to be expelled through the apical pores of the anthers. Recent studies have shown that the conditions in which buzz pollination occurs vary with respect to the floral morphology and vibration signals produced by the bees - mainly determined by the duration, amplitude, and frequency of the vibration. The structural topology and material properties of the flower also induce resonance and damping behaviour, thus mediating the transmission of substrate-borne vibrations. Along with the best-studied mechanism of buzz pollination*,* it is increasingly clear that vibroacoustic (VA) signals may have played a role in co-evolutionary responses that significantly impact several aspects of plant and insect ecology. However, studies on the material properties in terms of VA transmission are still in their infancy. Therefore, in this study, we numerically investigate the sensitivity of the floral topology on transmission of vibrations from a source signal. For this purpose, stamen structures at different scales and loading conditions are modelled and analysed in a finite element software package. A representative VA signal is implemented as the excitation at locations impacting the flower during feeding events. The results demonstrate that natural frequencies and mode shapes of the stamen may influence the conditions in which the vibrational energy is scattered across the flower. We also observe a frequency- and amplitude-dependent coupling which coincide with empirical observations.

Keywords: *Bombus terrestris,* bumblebee, Buzzing vibration, flowering plant, insect-mediated pollen transfer, vibration transmission

1. Introduction

To limit access to their pollen reservoir, many plants have developed specific floral characteristics. Bees and other insects requiring access to pollen use different types of vibrations that assist them in releasing pollen. Buzz pollination is the most common way to excite anthers, sac-like structures at the tip of the stamen (present in thousands of species [1]). This mechanism usually consists of a bee biting anthers or corollas with its mandibles and rapidly contracting its flight muscles (decoupled from the wings), generating vibrations that release pollen grains [2]. In the last decade, studies investigating buzz vibration in natural and agricultural environments are on the rise. From an evolutionary perspective, the use of buzz vibration offers a unique viewpoint to understand how plant and insect species have evolved to exhibit traits that promote co-dependence and survival. Certainly, such co-dependence cannot exist in vacuum. Studies that examined biomechanical feedback during feeding events discovered trends that may explain the macroevolution of buzz-pollinated floral morphologies [3]. One feature that seems to be the key in understanding of this evolution is the way the plants have adapted to their environment to proportionally dose the amount of pollen available to an individual floral visitor. Encapsulating the pollen within tube-like anthers preserves the pollen content against unconditional access, disadvantageous environmental influences, and pathogens. Small apical slits or pores at the tip of the anther allows efficient release of pollen to an individual floral visitor, promoting floral reproductive success [1]. More than 50% of the world's bee species, including carpenter bees (*Xylocopa* spp.) and bumblebees (*Bombus* spp.) use their mandibles and flight muscles to extract pollen from anthers by floral buzzing, a vibro-acoustic signal unique to each species [2,4-5].

The releasing of pollen depends on the floral morphology as much as it does on the vibratory feedback given by the bees. There have been attempts to characterise poricidal anthers—anthers with tiny pores that allow the pollen to be extracted rapidly when excited [3]; however, the dynamics of this phenomenon is understudied. The modal parameters of the stamen (e.g., natural frequency, damping ratio, and mode shape) may contribute to its movement during floral buzzing, thereby affecting the rate of pollen release. It is well known that exciting a structure at its resonant frequency amplifies the deformation it experiences under dynamic loading. Resulting pattern of deformation experienced by the vibrating anther affects how kinetic energy is transferred to the pollen grains and subsequently how the pollen grains exit the apical pore [4].

Besides physical experimentation, computational models could also be used to estimate the stamen's vibration modes, natural frequencies, and physical deformations under specified loading conditions [6]. The goal of this study is therefore to numerically investigate the propensity of the floral topology to efficiently transmit vibrations from a certain kind of source signal, or excited at a specific location. For this purpose, stamen structures at different scales and loading conditions are modelled and analysed using the commercial finite element software package. A representative bee signal is implemented as the excitation.

All studies are conducted on *Antirrhinum majus* (common snapdragon), a geographically widespread organism suitable to study pollination mechanisms. Even though the release of pollen in *A. majus* is not necessarily promoted by insect buzzing, still, we argue that vibrations induced by the bumblebee flight muscles may increase the efficiency in which the pollen is removed from the anthers.

2. Snapdragon – Bumblebee dynamics

Species of *Antirrhinum* display five petals arranged into an upper lip or two upper petals, and a lower or abaxial lip (three lower petals) that typically develops a palate occluding the mouth of the tube – see Fig. 1a. This mask-like palate of the flower is a sophisticated form of protection by forming a physical barrier. Small secretory structures, called nectaries, are often found at the base of all stamens, providing food (i.e., sugars) rewards for insect pollinators. Interestingly, this flower type is considered to have one

of the most occluded corollas among the angiosperms and displays an effective barrier between the nectar produced at the base of the flower tube and unwanted floral visitors [7]. Despite this, the physical barrier presented in this occluded corolla may not prevent all insects from entering the snapdragon flower to collect nectar and pollen.

Field surveys in natural conditions found flower visitation almost exclusively by 11 species of bees including *Apis mellifera* (honeybee) and *Bombus terrestris* (Buff-tailed bumblebee) [8]. This result covering the majority of *Antirrhinum* species suggests that large bees of the two long-tongued bee families are the major pollinators of *Antirrhinum*. *B. terrestris*, common and widespread in Europe and introduced (invasive) to Australia (Tasmania), New Zealand and Japan, is a generalist forager visiting a broad variety of flower types, and it readily incorporates novel plant species into its diet [1]. A tight snapdragon-bumblebee mutualism (Fig. 1b) has been investigated in detail over the last decades [8, 9]. In particular, the "*A. majus / B. terrestris*" - system of flower-insect interaction has been proposed as an efficient driver of flower evolution [10].
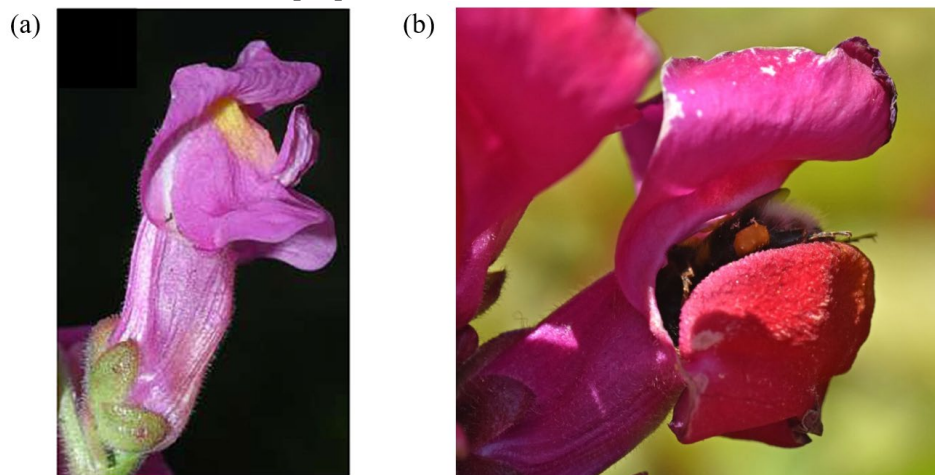

Figure 1: Snapdragon, or *Antirrhinum majus*. (a) complex morphology of the Corolla as close-up [11]; (b) a Buff-tailed bumblebee (*Bombus terrestris*) entering a snapdragon flower [12].

During bee entrance in *Antirrhinum*-like flowers, floral structures come in direct contact with the body of the vibrating insect. For instance, bees tend to grasp the filament in the stamen with their mandibles while curling their body around the anthers. Such direct contact between bees and floral structures could influence the vibration properties of the coupled bee-flower system. Figure 2 exemplifies how floral morphologies can differ in terms of their corolla, the number and shape of their stamens. These morphologies are often characteristic of their main pollination mechanism, such as buzz-pollination. In fact, distantly related plant species have convergently evolved similar structures when adapting to the same insect pollinators, and these recurring phenotypes are often referred to as pollination syndromes.

The vibrations produced by a bee during pollen collection can consist of a series of multiple short vibrations. The characteristics of the vibrations experienced in the anthers of a flower are also affected by the stiffness, mass, and material properties of the floral organs, as well as by the way in which a bee can excite the flower (e.g., by the wing muscles) [6]. The study in [13] investigated the variability in bumblebee pollination buzzes affecting the quantity of pollen released from flowers.

3. Material and methods

Inspired by the studies in [6], we developed a finite element model of the *A. majus* stamen from measured morphometric properties based on scanning electron microscopy [14]. A three-dimensional model of the stamen was created using CAD software. Figure 3 shows a simplified finite element model

of the ventral stamen, where filament and anther parts are visualised. The close-up look at the ellipsoid anther reveals cylindrical tubes representative of pollen sacs. The filaments of ventral and lateral stamens were measured to have the length of 25.4 mm and 19.9 mm, respectively, while the diameter of the filament ranging from 1.6 mm at the base to 0.73 mm at the anther connection. The material properties of the filament and anther were assumed to be identical to the study given in [6] for *S. elaeagnifolium*, i.e., $\rho = 154$ kg·m^{-3}, $E = 11.88$ MPa, $\nu = 0.45$ – values representative of soft biomaterials.
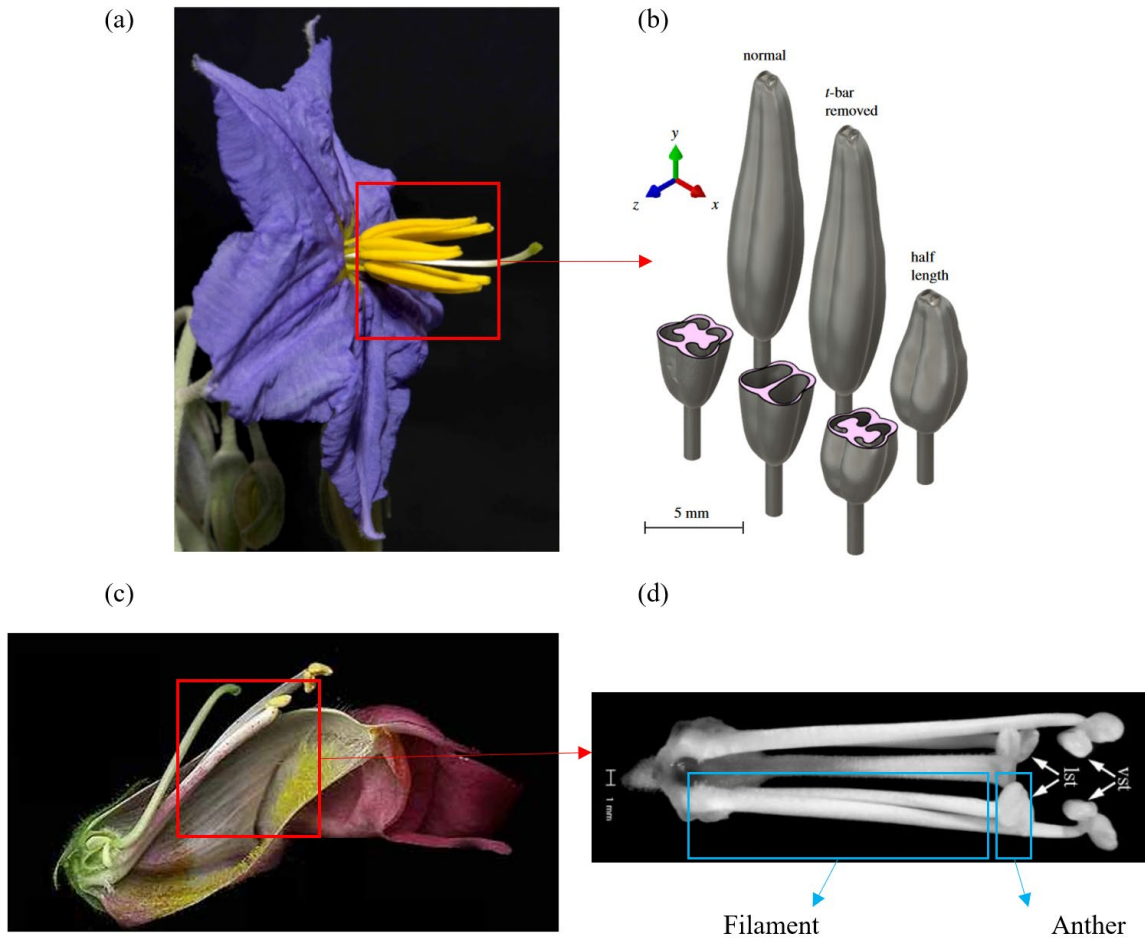

Figure 2: Images and cross-section of (a) *Solanum elaeagnifolium* [6] and (c) *Antirrhinum majus* flowers [15], respectively. (b) and (d) show the stamens with filament and anther parts [6,14]. Also, in (d) the relative size difference between lateral and ventral stamens is shown.

The mesh of the finite element model implemented in COMSOL Multiphysics (ver. 6.1) was discretised by 14,411 quadratic serendipity elements, which was sufficient for convergence of the low-frequency vibration modes. The degrees of freedom at the base of the filament were fully constrained to ensure a cantilever condition. The rest of the geometry was free to move. The first eight calculated vibration modes show a series of in-plane/out-of-plane modes, and torsional vibration mostly limited to the motion of the anther, in a frequency span of 50 Hz – 1.5 kHz (Fig. 4). As shown in Table 1, compared to *S. elaeagagnifolium*, the vibration modes of the *A. majus* ventral stamen are more flexible owing to a greater slenderness ratio of the filament. The lateral stamen experiences shift in the frequency of bending and torsional deflection due to different mass and stiffness distribution in the filament and anther.

Table 1: A comparison of undamped natural frequencies with respect to stamen sizes with- and across-species.

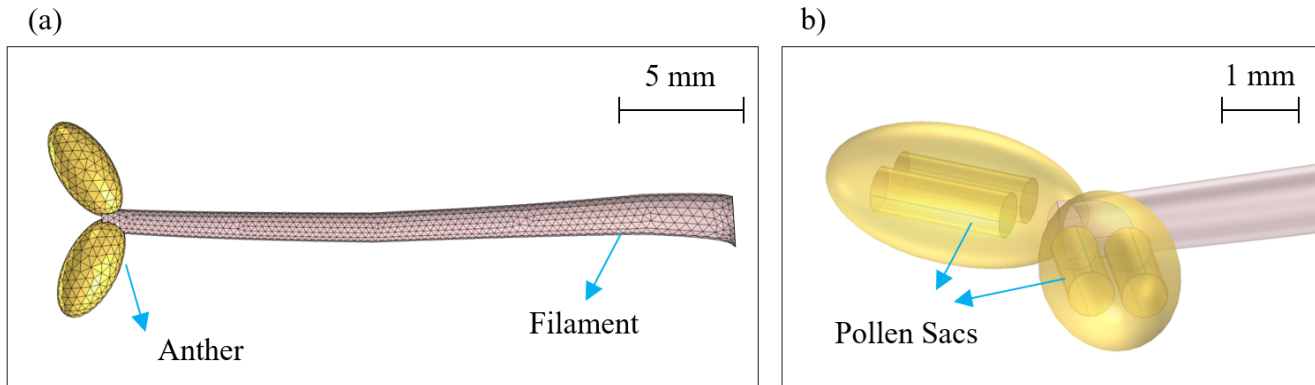|	Vibration mode frequency in [Hz]		
	S. elaeagagnifolium [6]	*A. majus* (ventral)	*A. majus* (lateral)
1st in-plane bending	63.8	53.1	47.3
1st out-of-plane bending	64.2	53.6	48.1
1st torsional	456.7	245.3	171.8
2nd in-plane bending	1212.5	331.6	307.7
2nd out-of-plane bending	1234.4	393.5	444.5
3rd in-plane bending	2425.0	771.1	797.7

(a)

b)

Figure 3: A simplified finite element model of the ventral stamen. (a) Tetrahedral mesh composition on the filament and anther; (b) a close-up of anther with cylindrical tubes representing pollen sacs.

We have implemented a sinusoidal source signal to calculate the frequency response at the tip of the stamen at different loading conditions. The mass of the bee ($m_b = 150$ mg [6]) and the excitation through mandibles of the bee was set to act upon the filament at different locations: A1, A2, A3, denoting 50% filament length, 75% filament length, and 50% filament length for double contact surface, respectively. The applied force was in the symmetry axis of the *yz*-plane with a magnitude of 3.4 μN [14], in the frequency range of 0 Hz to 1,500 Hz. The results comparing the damped response (for damping ratio of $\zeta = 0.05$ [6]) at the tip of the anther around the sac-tubes are shown in Fig. 5. Due to the mass loading caused by the bee upon the filament (almost 20:1 ratio), we see a considerable shift in the frequency spectra with many more modes appearing in the low-frequency region. At the dominant frequency bands of a typical excitation signal whose 1st and higher harmonics are shown in Fig. 5b, transfer frequency response function obtained through numerical simulation also shows distinct peaks that are within the nominal frequency range of 264 Hz to 386 Hz, representative of the buzzing vibration of a *B. terrestris* worker [13]. The peak frequency of 324 Hz ± 3.67 Hz is shown to coincide with the vibration peak observed in case A2, where the mass loading/forcing is near the anther on the filament.

The modal density of a mass-loaded stamen is much higher in the low-frequency region to support different anther movement at a given excitation location. Evidenced by A1, A2 and A3 cases in Fig. 5b, the anther can experience local vibration amplification due to vibration transmission through the stamen and the excitation at the base of the flower.
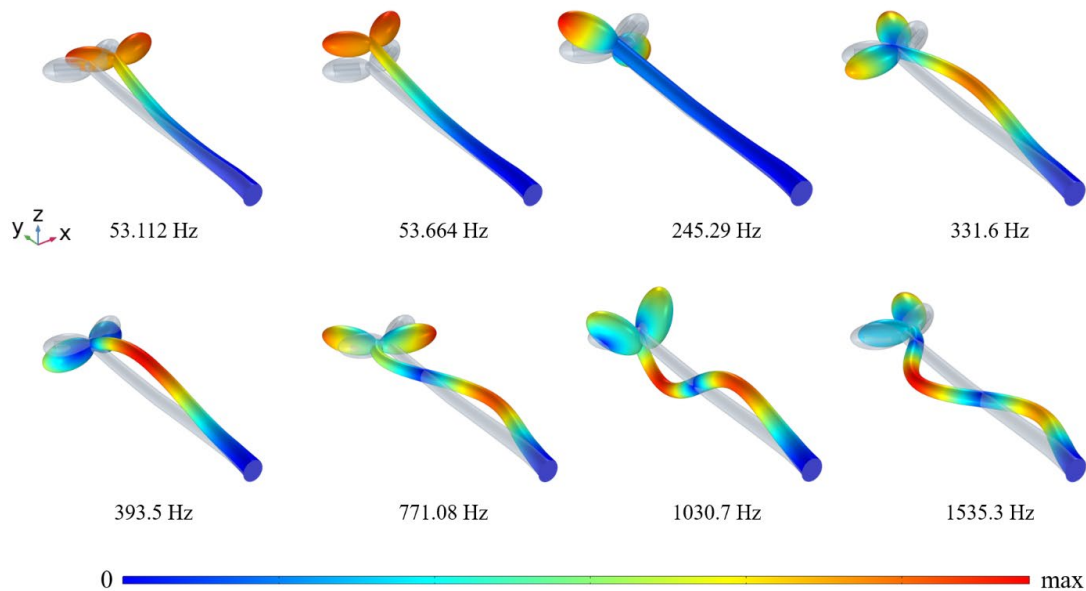
Figure 4: *Antirrhinum majus* flower ventral stamens. First undamped eight vibration modes are shown. The deflections from the neutral position (in grey) are not to scale.

4. Discussion

Haptic feedback, airborne and substrate-borne vibrations, and how they may be employed to benefit insects, e.g., in foraging, courtship, alarm and eavesdropping against predators or competitors is well studied [16-18]. Several Hymenopterans and Isopterans show a great efficacy of using such signals, e.g., waggle dance in honeybees [19]. Interdisciplinary studies investigating the buzz-pollination phenomenon have shown a closely intertwined relationship between buzz-pollinating flowers and efficient pollinators such as bumblebees. The role of pollinators driving morphological diversity has been recognised for over two centuries [8]. Evolution of the floral morphology in response to environmental factors and resulting reward strategies suggest that vibroacoustic signals may also be the driving factor in plant-insect communication.

In pollination of *Antirrhinum*–like flowers, haptic feedback by the bumblebees is the first and foremost factor. When the bee lands on the bottom lip of the flower, its weight (in relation to a spring-like mechanism) creates an opening which the bee can use to get inside the flower. From this example, we can gather that floral morphology might have evolved in such a way to promote biomechanical feedback at proportionately applied mass loading and excitation amplitude. Especially for *A. majus*, the closed mechanism of the corolla requires a force greater than a certain threshold to allow access to the pollen. Through comparative experimental and numerical studies, we may be able to find out what this threshold may represent in the form of plant-insect-physiology ratios and in relation to plant-insect pollination dynamics.

Based on the examples of *Solanum–Bombus* spp. [4,6], the frequency, amplitude and location of buzz vibration may have a dominant effect on the rate of pollen release. Even though *A. majus* is not among the buzz-pollinated plant species, it is argued that similar buzz vibrations induced by the flight muscles in the thorax of the bee may increase the efficiency in which the pollen is removed if the stamen is excited at locations coinciding with anti-nodal positions of anther deformation (as shown in Figure 5b).

In the case of buzz-pollinated species, lab studies using artificial vibrations between 450 Hz and 1,000 Hz show twice as much pollen removal compared to vibrations of under 400 Hz [20]. It is not clear why this would be the case as the spectrogram of a typical buzz-pollination signal shows 1st harmonics that are in the range of 240 Hz – 405 Hz [1]. This suggests that higher harmonics and amplitude nonlinearity

in the signal may play a factor in pollen removal in combination with the structural characteristics of the stamen.

To model the complex dynamics of flower-bee interactions, the material properties and the material model in relation to internal structures/geometries are likely important. Local stiffnesses and densities in the corolla as well as the stamen, and the excitation signal are contributing factors that require identification in explaining pollination dynamics.
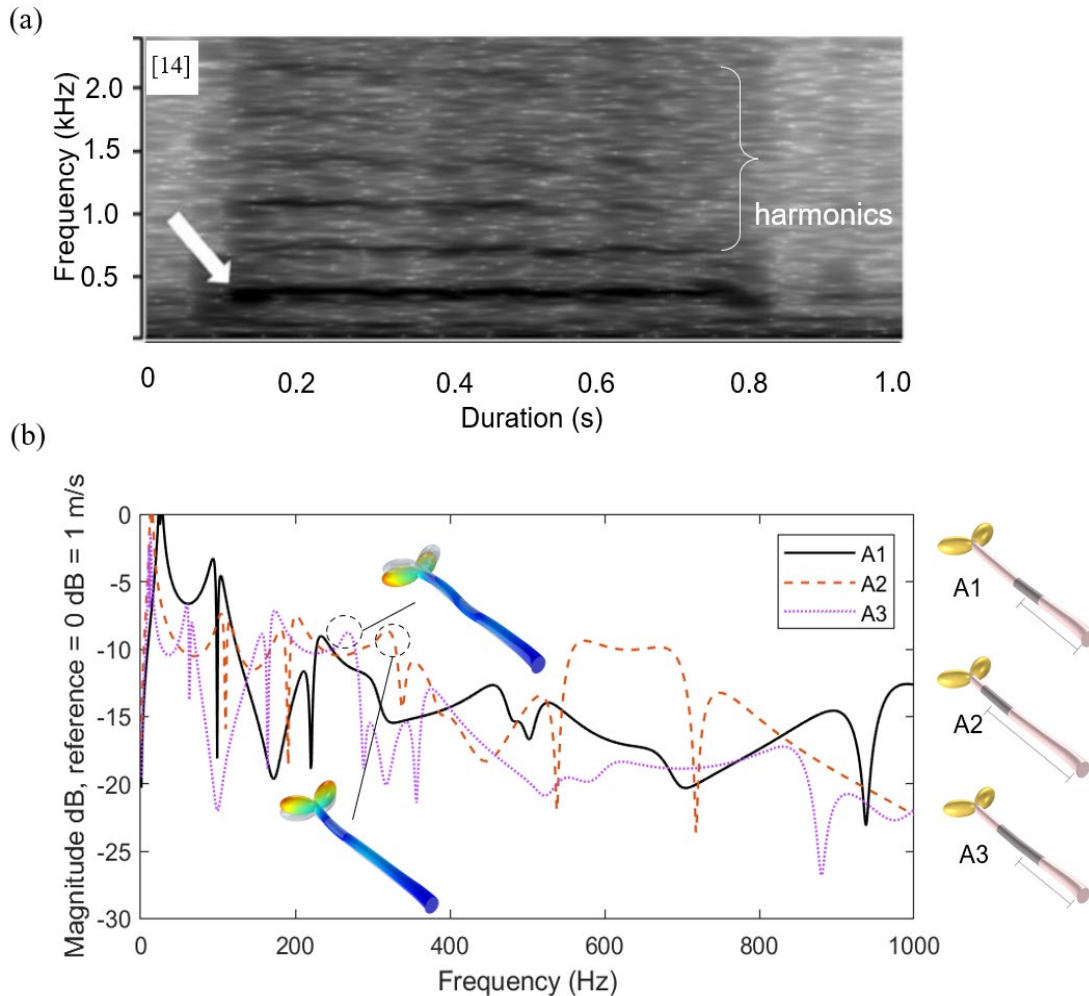

Figure 5: Transfer function of *A. majus* flower ventral stamen under different loading conditions. (a) A spectrogram of a typical buzz-pollination signal by a *Bombus terrestris* worker [13]; (b) comparison of damped response at the tip of the anther for the loading conditions A1, A2, and A3. Grey-shaded area demonstrates the surface loading due to the mass of the bee. The response profile shows the tip movement at the resonance frequencies 269 Hz, and 323 Hz for A3 and A2, respectively. These frequencies are within the 'buzzing range' of bumblebee, i.e., 264 Hz – 386 Hz, with peak frequency of 324 ± 3.67 Hz [13].

5. Concluding remarks

In this study, we have investigated the dynamics of a bumblebee feeding event in a   snapdragon flower via numerical modelling and analysis. The results have shown that the geometry and material properties of the stamen structure, and mass loading/excitation profile of the bumblebee may result in a special coupling condition to enable strong interaction at the anther that allows for efficient release of pollen. These findings may explain the robustness of the biomechanical feedback through various plant and insect species. A flower model updating procedure through μCT scan and live experiments are

currently planned to conduct bioassays and validate our findings through numerical analysis. This model should also be compared to those generated in buzz-pollinated flowers, such as *Solanum* spp., to understand the different mechanisms for pollen release present in insect-pollinated angiosperms.

Acknowledgments

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