ACTIVITY OF ASYNCHRONOUS FLIGHT MUSCLE FROM TWO BEE FAMILIES DURING SONICATION (BUZZING)

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Summary

The indirect flight muscles of bees are used to produce a variety of actions in addition to flight, including sonication, which has a higher frequency than flight. We observed the dynamic movement of the scutum during sonication and the transition from tethered flight to sonication. During sonication, the scutum oscillated above its rest position, indicating that the conformation of the structural components of the thorax had been altered. Sonication vibrations of the thorax occurred by deformation of the scutum rather than by opening of the scutal fissure and are smaller than vibrations associated with flight. During tethered flight, the ratio of muscle activity (recorded *via* electromyograms) between the dorsal longitudinal muscles and the dorsoventral muscles approached 1, but during sonication the ratio was significantly higher (up to 4.0). This increase may cause the dorsal longitudinal muscles to contract further than the dorsoventral muscles and close the scutal fissure during sonication, so limiting the displacement of the wings and 'decoupling' them from the indirect flight muscles.

Key words: *Bombus occidentalis*, bumblebee, buzz, carpenter bee, flight muscle, neural control, sonication, *Xylocopa californica*, *Xylocopa varipuncta*.

Introduction

The large indirect flight muscles of bees are used to produce a variety of actions in addition to flight. These include sonication for pollen collecting, for defence and for compacting soil within nests, all of which have higher frequencies than flight (Buchmann, 1983). During flight, the antagonistic actions of the indirect flight muscles cause the scutal fissure to open and close, producing a lever action of the wings about the pleural wing joint (Fig. 1). The motor inputs to each of the indirect flight muscles are not in a 1:1 relationship with the muscle contractions, and these muscles are termed fibrillar (asynchronous) muscles. The high oscillation frequency is maintained by stretch activation of the antagonistic muscles at the natural frequency of the system (Machin and Pringle, 1959). The natural frequency is the frequency at which an undamped system will vibrate in the absence of an opposing force. The sonication frequency of bees is usually significantly higher than the flight frequency (King, 1993), presumably as a result of the reduced inertia of the flight system when the wings are decoupled, which will raise the natural frequency. Heinrich (1993) discussed mechanisms by which the wings may be decoupled from the indirect flight muscles and wing motion be eliminated despite contraction of those muscles. These included the use of the third axillary muscle to fold and decouple the wings and closing of the

mesonotal suture so that movement of the indirect flight muscles is prevented. Roeder (1951) found that the frequency of neural excitation of the indirect flight muscles of several insects with fibrillar flight muscles did not vary when the wings were amputated, even though the 'wingbeat' frequency increased by up to 100 %, indicating that the frequency increase may be due to inertia effects alone. However, in a series of experiments in which the inertia of the wings was altered by clipping or adding masses to them, Sotavalta (1952) found that frequency was proportional to $I^{-0.37}$ rather than to $I^{-0.5}$ as expected, where I is the wing inertia. It is therefore possible that other effects, such as the excitation rate of the indirect flight muscles, could affect the natural operating frequency of the system. Also, sonication is not necessarily the same as flight with amputated wings; in particular, sonication occurs in pulses of short duration (approximately 0.1 s) (King, 1993).

Esch and Bastian (1968) found that the wingbeat frequency of honeybees increased with the frequency of motor spikes, so it is possible that frequency differences between flight and sonication are related to the muscle activation frequency. Esch *et al.* (1991) found in bees that, during shivering, a higher excitation rate of the dorsal longitudinal muscles (DLMs) caused unequal shortening of the DLMs relative to the
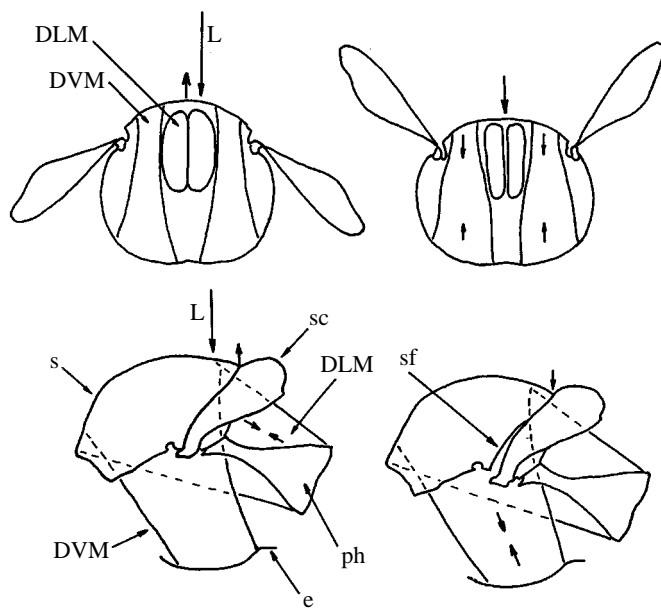
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Fig. 1. Mechanism of wing movements by indirect flight muscles (from Snodgrass, 1956). Upper drawings are schematized transverse sections through the thorax; lower drawings show lateral views of the thorax, anterior to the left, with part of the thorax removed to show the dorsoventral muscle. Left-hand drawings show dorsal longitudinal muscles contracted, scutal fissure closed, scutum deformed slightly and wings depressed. Right-hand drawings show dorsoventral muscles contracted, scutal fissure open, scutum flattened and wings lifted; DLM, dorsal longitudinal muscle; DVM, dorsoventral muscle; e, episternum; L, position of laser displacement meter used to measure thoracic displacement; ph, postphragma; s, scutum; sc, scutellum; sf, scutal fissure. Unlabelled arrows show displacements.

dorsoventral muscles (DVMs), which forced the structures connected to the mesophragma against a mechanical stop and limited the wing oscillations. Although they were investigating shivering, which they showed did not involve stretch activation of the indirect flight muscles, a similar mechanism may apply during sonication, in which stretch activation does occur.

We report here an investigation into the neural excitation of the indirect flight muscles during tethered flight and alarm sonication and during the transition between these activities. Thorax displacements during sonication were measured using a non-contact transducer.

Materials and methods

Bombus terricola occidentalis (Greene) workers and *Xylocopa varipuncta* (Patton) and *X. californica arizonensis* (Cresson) queens were collected from the field, stored in an insulated container with ice and used within 8 h. In order to obtain representative acoustic recordings of flight and sonication for these species, their respective acoustic frequencies were determined by tape-recording free and tethered flight and alarm sonication in a controlled-environment sound-attenuating room. To determine the relationship between the magnitudes and frequencies of the

vibrations generated during tethered flight and sonication, the bees were tethered by the episternum to a steel post using molten beeswax. The bees were induced to fly by suddenly removing their tarsi from a cardboard substratum and simultaneously blowing air onto the front of the head. Alarm sonication was initiated by pinching a tarsus with forceps. A microphone (Brüel and Kjær 4134) was held approximately 50 mm from the flying or sonicating bees and the sound was tape-recorded for analysis as described below. Thoracic displacements were also measured using a laser displacement meter (Keyence LB72) aimed at the scutum, slightly behind the scutellar fissure (Fig. 1). The signals were recorded using a computer data acquisition unit (Strobes Engineering Ltd, 901A) for later analysis.

Silver electrodes, 110 μm in diameter and coated with Teflon except at the tips, were inserted into the DVM and DLM of cold-anaesthetised bees. Ground electrodes were inserted deep into the thorax and all wounds were sealed with wax. The bees were tethered by the notum to a metal post using wax. They were then gradually brought up to room temperature (25 °C) and allowed to acclimatise, so that they were 'flying' vigorously, before recordings were taken. A microphone (Brüel and Kjær 4134) was positioned approximately 50 mm above the bee to record flight or sonication frequencies. A separate microphone was used for an observer to record the times when a bee began flight or sonication. The electrodes were connected to an a.c. amplifier (AM systems 1700), and electromyograms (EMGs) were recorded simultaneously with the two microphone channels on an instrumentation tape recorder (Vetter, model E). Selected portions of the EMG and acoustic records were digitised and stored on computer (Axotape) at a sampling frequency of 3300 Hz. Synchronisation of the two EMGs was tested using frequency phase analysis (Strobes Engineering Ltd, PC-acquisition).

EMG events were counted for each muscle during flight and sonication. The DVM and DLM each contain an unknown number of motor units and the complexity of the recorded pattern when more than one of the units are nearly synchronously activated made it difficult to identify single action potentials. EMG events were therefore counted as single or grouped action potentials which were +10 dB above the background noise level and which were separated by a 0.005 s quiescent period. Events which were counted from a typical recording are indicated by asterisks on Fig. 2.

Results

Table 1 shows the acoustic frequencies measured during free and tethered flight and sonication; the range of frequencies during free and tethered flight overlapped but the sonication frequency was higher, so that discrimination of the activities was possible for all species. The recordings of scutum movement during sonication showed that oscillation of the scutum was mainly above the rest position; Fig. 3A shows the upward displacement of the scutum of *X. varipuncta* during a typical sonication. Just before sonication
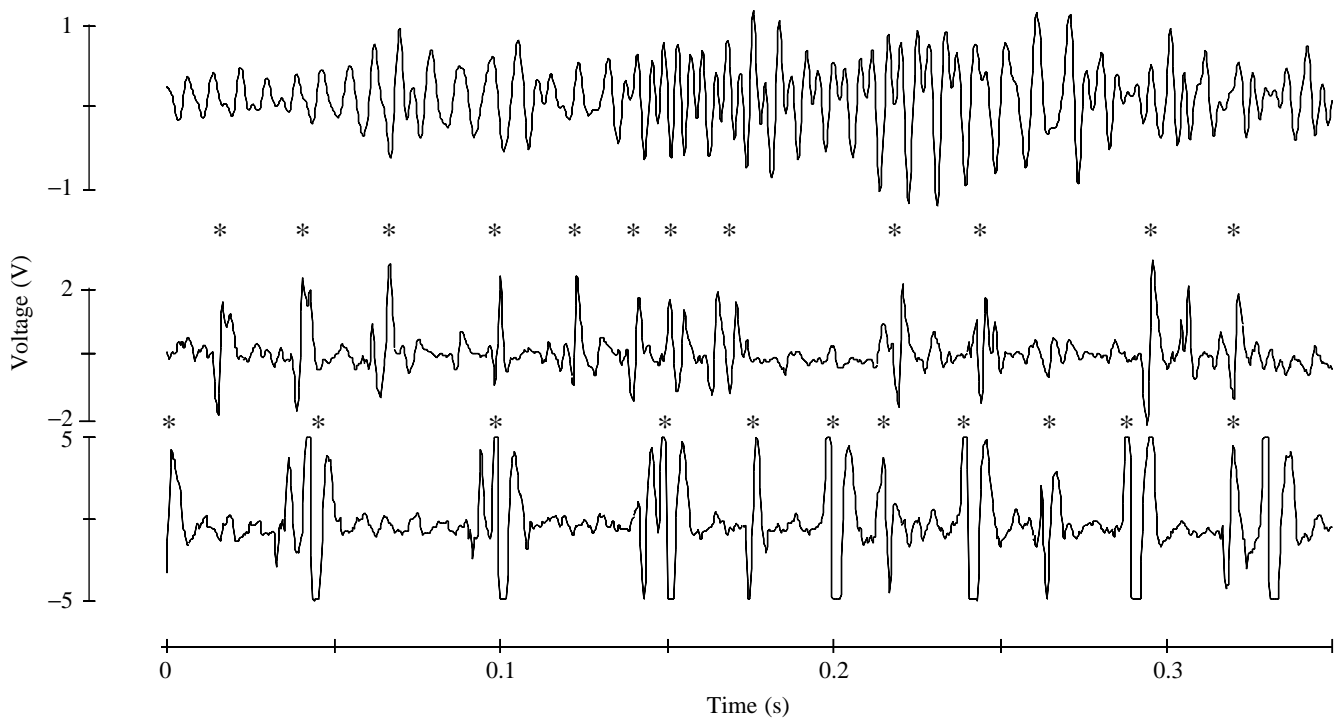
Fig. 2. Acoustic (upper trace) and electromyography recordings from the dorsoventral muscle (DVM, middle trace) and dorsal longitudinal muscle (DLM, lower trace) from *Xylocopa varipuncta* changing from tethered flight (time 0–0.1 s; wingbeat frequency 120 Hz) to a short sonication (time 0.15–0.25 s; vibration frequency 240 Hz). The ratios of DLM/DVM excitation during flight and sonication were 0.9 and 2.5, respectively. Note that the DLM record was 'clipped' owing to over-amplification. Asterisks indicate the EMG events which were counted (see Materials and methods).

began (at 0.015 s), the scutum was raised above the rest position, probably by contraction of the DLM, which would close the scutal fissure and distort the scutum slightly upwards (see Fig. 1). Fig. 3B shows the displacement during quiescence, a short burst of tethered flight and then sonication. The displacement during tethered flight was both above and below the quiescent position, while the displacement during the subsequent sonication was again mainly above the quiescent position.

Simultaneous EMG recordings from the indirect flight muscles and recordings of acoustic signals allowed identification of the motor patterns underlying tethered flight and sonication. A high frequency and synchronisation of excitation to the two muscle groups was found at the start of tethered flight. After flight had been established, the frequency

of excitation to the DLM ranged from 10 to 35 EMG events per second, and we did not observe synchronisation or predictable phase relationships between the DLM and DVM motor patterns. The mean DLM/DVM excitation ratio during tethered flight was 1.12±0.21 (S.D., N=8). Table 2 shows the DLM/DVM excitation frequency ratio ranges for the species investigated.

We did not observe synchronisation or predictable phase relationships between the DLM and DVM motor patterns at the transition between tethered flight and sonication (e.g. see Fig. 2) or between short pulses of sonication; for a series of sonication pulses, the motor pattern during the period between the pulses did not appear to differ from that during the pulses. When tethered flight changed to sonication without a quiescent period between these activities, the transition was typically very rapid, occurring within a single wingbeat cycle (Fig. 2, 0.14 s). When short pulses of sonication occurred, similar to the pattern occurring during pollen sonication (King, 1993), muscle activation was continuous during the quiescent periods and small acoustic vibrations (approximately 10 % of the peak amplitude during sonication) occurred. Excitation occurred during the quiescent periods leading up to tethered flight and between flight and sonication, although it often ceased when a flight or sonication period ceased. The mean ratio between excitation of the DLM and DVM during sonication for all species observed was 2.5±0.9 (S.D., N=6), which was significantly higher than that during tethered flight (P=0.0046).

Table 1. *Ranges of acoustic frequency during flight and sonication*

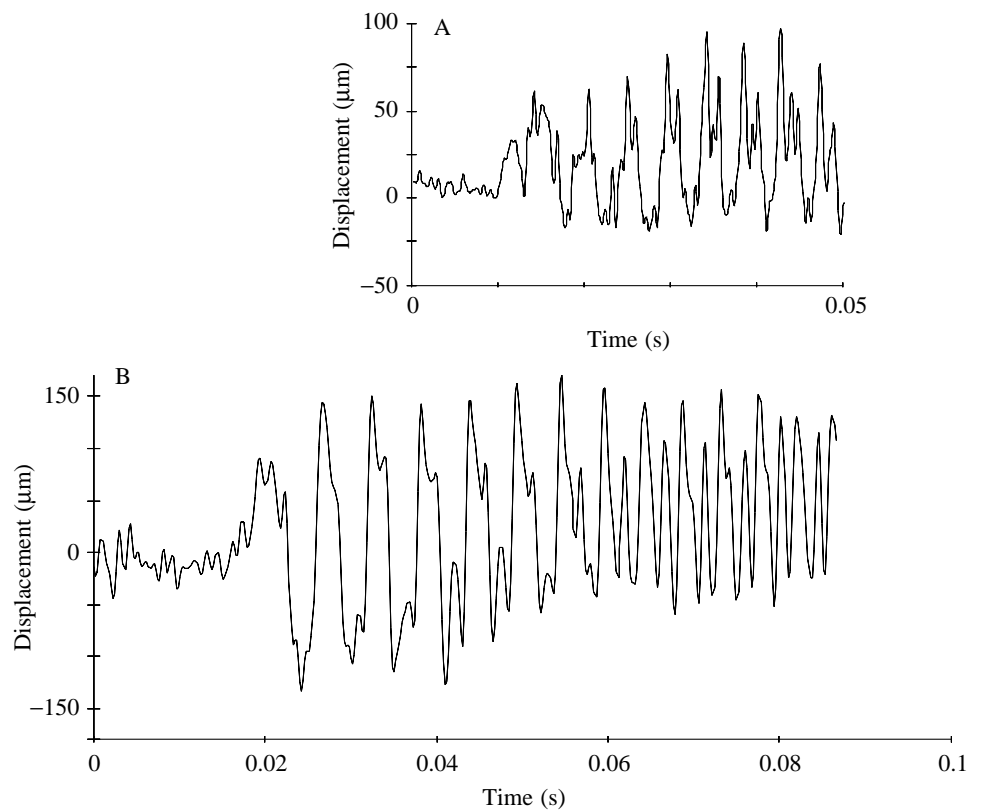| Activity	Acoustic frequency (Hz)		
	Bombus occidentalis	*Xylocopa californica*	*Xylocopa varipuncta*
Free flight	126–187 (3)	121–122 (2)	119–126 (2)
Tethered flight	112–136 (4)	107–147 (3)	119–141 (3)
Sonication	253–371 (3)	197–297 (7)	224–249 (3)

N is given in parentheses.

Fig. 3. Displacement of the scutum of a *Xylocopa varipuncta* female during flight and sonication. (A) Quiescence (0–0.01 s) followed by sonication (0.015–0.05 s). (B) Quiescence (0–0.015 s) followed by a short period of flight (0.02–0.05 s) followed by sonication (0.06–0.09 s). Note that during flight the displacement is above and below the quiescent position, but during sonication it is mainly above the rest position.

Discussion

The laser displacement meter used in this study allows quantitative dynamic displacements of the thorax to be measured directly. Such displacements were previously measured indirectly by following the rotation of a light beam reflected by the scutellum (Esch and Goller, 1991). During sonication, dynamic scutum displacement was mainly above the rest position (Fig. 3); the scutal fissure would thus be closed (see Fig. 1) and deflections would mainly be due to elastic deformation of the thorax. During pollen sonication, bees assume a characteristic 'C' posture (Buchmann and Hurley, 1978; King, 1993), which may improve contact between the episternum and flower anthers, but may also be a result of contraction of the DLM beyond the rest position, resulting in the thoracic sclerites being forced against the mechanical stop formed by the closure of the mesonotal suture

Table 2. *Ranges of DLM/DVM excitation frequency ratio*

	DLM/DVM excitation frequency ratio		
Activity	*Bombus occidentalis*	*Xylocopa californica*	*Xylocopa varipuncta*
Flight	1.0–1.6 (2)	1.0–1.2 (2)	1.0–1.2 (4)
Sonication	2.4–4.0 (3)	1.5 (1)	1.7–2.0 (2)

DLM, dorsal longitudinal muscle; DVM, dorsoventral muscle.

Excitation frequency is the number of EMG events per second (see Fig. 2).

N is given in parentheses.

(Esch *et al.* 1991). Vibration of the thorax with the scutal fissure closed would require deflection of the thoracic sclerites (in particular the scutum), which is presumably why the range of scutum displacement measured during sonication was smaller than that during tethered flight. The scutum undergoes dynamic deflection during both flight (Snodgrass, 1956) and sonication, although displacement during flight occurs as a result of both opening of the scutal fissure and distortion of the scutum.

The mean DLM/DVM excitation ratio during tethered flight of 1.12 was similar to the value of 0.86 reported by Esch and Goller (1991); from their Fig. 4, the excitation frequency during flight was also similar (DVM excitation frequency was approximately 35 Hz). The high-frequency excitation that Esch and Goller (1991) observed at the initiation of flight was also observed in the present study during the initiation of flight but did not occur at the transition to sonication or between short-duration sonication pulses. The excitation frequency of the DLM was higher than that of the DVM during sonication for all of the bees investigated. Between the short-duration sonication pulses, the acoustic vibrations did not completely disappear and the intermittent muscle group activation was maintained throughout; pulsed sonication therefore appears to be produced by regularly varying the magnitude of the thoracic vibrations without a regular variation in the DVM or DLM motor pattern.

The excitation frequency during sonication was higher than that found by Esch and Goller (1991) for shivering bees. During shivering, the higher excitation rate of the DLM

caused unequal shortening of the DLM relative to the DVM, which forced the structures connected to the mesophragma against a mechanical stop formed by the closure of the mesonotal suture and limited the wing oscillations. In the present study, the DLM/DVM excitation ratio during sonication was significantly higher than during tethered flight, which could also result in relatively greater shortening of the DLM and hence activate the mechanical stop. Although regular thoracic vibrations do not occur during shivering, muscle excitation during sonication may cause stretch activation and subsequent vibrations to occur, regardless of the closing of the scutal fissure and the mechanical stop. Vibration during sonication would then occur by elastic deformation of the thorax in manner similar to warm-up shivering, rather than by opening of the scutal fissure as occurs during flight. However, thoracic motion produced during shivering bears little resemblance to the regular vibrations during sonication, which are more regular and vigorous and at a constant high frequency (King, 1993). The complex waveforms typical of DVM activity during sonication could be a result of a higher firing frequency of all the motor units of the DVM or a lower firing frequency of single motor units at particular phases with respect to each other; our data do not allow us to differentiate between these possibilities. Esch and Goller (1991) investigated buzzing in honeybees and bumblebees during warm-up shivering and found that it was started by a sudden burst of activity (of approximately 200 Hz) in at least three groups of DLM units, which resulted in a more rapid DLM contraction. However, the excitation frequencies reported here for sonication were lower than the values of Esch and Goller (1991) for the short-duration (three vibration cycles) buzzing associated with warm-up shivering (the maximum frequency measured was approximately 60 Hz).

Indirect flight muscle systems operate at the natural frequency of the flight system (Machin and Pringle, 1959). If the main difference between flight and sonication is reduced inertia due to decoupling of the wings from the thorax, then it would be expected that the motor patterns during flight and sonication would be similar. The higher DLM/DVM excitation frequency ratio during sonication compared with tethered flight and the resulting presumed closure of the scutal fissure (which will decouple the wings from the indirect flight muscles) may alter the vibration characteristics of the thorax such that the flight system will vibrate at a higher frequency. Structures may be forced to vibrate in various modes by clamping at nodal points (for example, stringed musical instruments). If the vibration mode at the sonication frequency has a vibration node near the scutal fissure, then wing motion during sonication would be small.

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