

J. W. Dawson · F.-H. Leung · R. M. Robertson

## Acoustic startle/escape reactions in tethered flying locusts: motor patterns and wing kinematics underlying intentional steering

Received: 29 September 2003 / Revised: 29 March 2004 / Accepted: 30 March 2004 / Published online: 29 April 2004  
© Springer-Verlag 2004

**Abstract** We simultaneously recorded flight muscle activity and wing kinematics in tethered, flying locusts to determine the relationship between asymmetric depressor muscle activation and the kinematics of the stroke reversal at the onset of wing depression during attempted intentional steering manoeuvres. High-frequency, pulsed sounds produced bilateral asymmetries in forewing direct depressor muscles (M97, 98, 99) that were positively correlated with asymmetric forewing depression and asymmetries in stroke reversal timing. Bilateral asymmetries in hindwing depressor muscles (M127 and M128 but not M129) were positively correlated with asymmetric hindwing depression and asymmetries in the timing of the hindwing stroke reversal; M129 was negatively correlated with these shifts. Hindwing depressor asymmetries and wing kinematic changes were smaller and shifted in opposite direction than corresponding measurements of the forewings. These findings suggest that intentional steering manoeuvres employ bulk shifts in depressor muscle timing that affect the timing of the stroke reversals thereby establishing asymmetric wing depression. Finally, we found indications that locusts may actively control the timing of forewing rotation and speculate this may be a mechanism for generating steering torques. These effects would act in concert with forces generated by asymmetric wing depression and angle of attack to establish rapid changes in direction.

**Keywords** Avoidance · Electromyography · Flight · High-speed cinematography · Ultrasound

**Abbreviations** ASR: acoustic startle response · dB SPL: decibel sound pressure level (re: 20  $\mu$ Pa RMS) · EMG: electromyogram · FWA: forewing asymmetry · HWA: hindwing asymmetry

### Introduction

When tethered (stationary) flying locusts are stimulated acoustically with trains of short duration, high-frequency sound pulses, they respond with an acoustic startle response (ASR) (Hoy et al. 1989; Robert 1989) that consists of head rolling away from the stimulus side, hindleg deflection, abdomen deflection and dorsiflexion, changes in wing beat frequency (including flight cessation) and changes in wing stroke kinematics (Robert 1989; Robert and Rowell 1992; Dawson et al. 1997). These changes are accompanied by the production of yaw torques away from the stimulus and it is presumed that these changes are capable of changing the flight path of free-flying individuals (Robert 1989; Robert and Rowell 1992). Dawson et al. (2004) have confirmed that free-flying locusts react to synthesized sounds with turns, loops and spirals, drops to the ground, and rapid ascents. Postural changes, such as leg and abdomen deflection are indicative of steering attempts for tethered, flying locusts (Camhi 1970); however, changes in wing kinematics are what is primarily responsible for generating the aerodynamic force necessary for changing direction (see reviews by Taylor 2001 and Dudley 2002). In this paper, we have used an acoustic stimulus to induce intentional steering responses in tethered, flying locusts and simultaneously measured the resulting changes in wing kinematics and depressor muscle activation. We show that the motor pattern recorded from forewing and hindwing direct depressor muscles correlates with key changes in wing kinematic parameters observed during steering attempts.

The literature on the neuromuscular mechanisms underlying steering in locusts primarily describes correlations of muscle activity, wing kinematics and postural changes for maintaining a straight course of flight in the

J. W. Dawson · F.-H. Leung · R. M. Robertson  
Department of Biology, Queen's University,  
K7L 3N6 Kingston, Ontario, Canada

J. W. Dawson (✉)  
Department of Zoology,  
University of Cambridge, Downing Street,  
Cambridge, CB2 3EJ, UK  
E-mail: jwd30@cam.ac.uk  
Tel.: +44-1223-334455  
Fax: +44-1223-336676

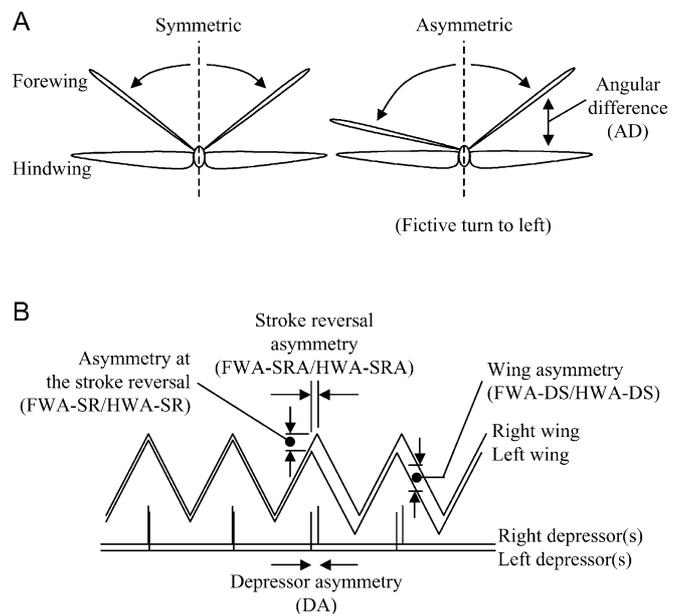
face of environmental perturbation and non-linearities in the flight machinery (Burrows 1996). However, intentional steering (e.g. to avoid a predator) is different than steering to maintain a straight course of flight (correctional steering) in several ways. Wing kinematic changes and postural adjustments during intentional steering are frequently larger relative to those seen during correctional steering (Robertson and Reye 1992; Robertson et al. 1996; Dawson et al. 1997). Abdomen deflection is effected by different segments during intentional steering (Camhi 1970). During intentional steering to auditory stimuli, the direction of head roll is opposite to that during optomotor steering (Robert and Rowell 1992). Finally, intentional steering differs from correctional steering in requiring suppression of the 'autopilot'. This means that differences must exist in the motor pattern and in the operation (and possibly organization) of the neural circuitry responsible for establishing the motor pattern.

From correctional steering studies, recordings from flight muscles show that the changes that occur during steering are in recruitment, burst length (spikes per burst) and phase (time of depolarization) of muscle activation (for reviews see Rowell 1988; Burrows 1996). Changes in recruitment of motor fibres and in spikes per burst affect the force of muscle contraction and indirectly affects the timing of wing movements. These studies show that shifts in the timing of contralaterally homologous muscle depolarizations are most sensitive to changes in yaw, pitch and roll (Zarnack and Möhl 1977). Changing the relative time of activation of contralateral muscles can bring about asymmetries in the behaviours of left-right wing pairs, and, depending on the effect the muscles exert on the wings, this can have aerodynamic consequences. During intentional steering, where large shifts in aerodynamic force are required, we expect large asymmetries in contralaterally homologous muscle timing.

Zarnack and Möhl (1977) recorded motor patterns of locusts flying in a laminar wind stream while being rolled, pitched and yawed; with the three flight axes being manipulated in isolation. They recorded from the six direct depressor muscles of the fore- and hindwings on each side of the locust and found that many of the muscles shift together. Thüring (1986) found similar shifts in depressor muscles in response to movement of an artificial horizon and noted that the shifts were correlated with the production of roll torques. Phase shifts in the time of activation of forewing and hindwing muscles on one side of the insect and in the time of activation of muscles within a single wing also occur and do affect wing kinematics. The time of forewing depression and elevation relative to the hindwing will affect the flow of air across the wing pairs, and in this way, as suggested by Wilson (1968), the forewings may act as leading-edge flaps for the hindwings. The angle of attack of a wing, which is critically important for generating lift, is established by the relative timing of muscles within the wing (Nachtigall 1989).

Dawson et al. (1997) showed that during intentional steering, the wing kinematic changes that occur in locusts responding to auditory stimuli include the production of asymmetries in the elevation angles of the forewings during the downstroke. They argued that the aerodynamic significance of asymmetric wing depression was to tip the direction of the lift produced by the left and right wing couple toward the side of the locust with the lower wing (Dawson et al. 1997). Further, it was suggested that these effects would add to the aerodynamic effects of changes in angle of attack, wing beat frequency and body posture (e.g. abdomen and leg deflection) to augment steering torque production. These suggestions are still to be confirmed experimentally but support for asymmetric wing depression being of aerodynamic significance would come from correlations of motor patterns and the kinematic changes that establish asymmetric wing depression.

Using high-speed cinematography, Dawson et al. (1997) found that asymmetric depression of the forewings was correlated with asymmetries in the timing of the stroke reversal and that forewing asymmetry was accompanied by large shifts (larger than typically observed during correctional steering) in forewing first basalar activity. This study proposed a model relating depressor muscle timing and asymmetric wing depression (Fig. 1). This model predicts that asymmetric wing



**Fig. 1** A Schematic diagram of a locust, viewed from behind during the downstroke, in straight flight (*left*) and during a turn to the left (*right*). During straight flight the wing strokes are symmetrical during the downstroke but become asymmetrical during a turn to the left. B A model predicting that asymmetric depression of the wings results from bulk shifts in the timing of depressor muscles which bring about changes in the timing of stroke reversals. In the schematic above, left direct downstroke depressor muscles fire before the right depressor muscles bringing about an earlier stroke reversal of the left wing relative to the right. The result is that the left wing is depressed more than the right wing throughout the downstroke but not during the upstroke. Modified from Dawson et al. (1997). The kinematic measurements illustrated and labelled are explained in Table 1

depression is established by the relative timing of the stroke reversals of the left and right wings. In the model, the left and right wings are elevated symmetrically but differences in the timing of the left and right depressor muscles cause one wing to reverse direction before the other thereby generating asymmetric wing depression. Since stroke reversal timing is determined by depressor muscle activity, the prediction from this model is that bulk shifts in several direct depressor muscles would be observed during intentional steering responses.

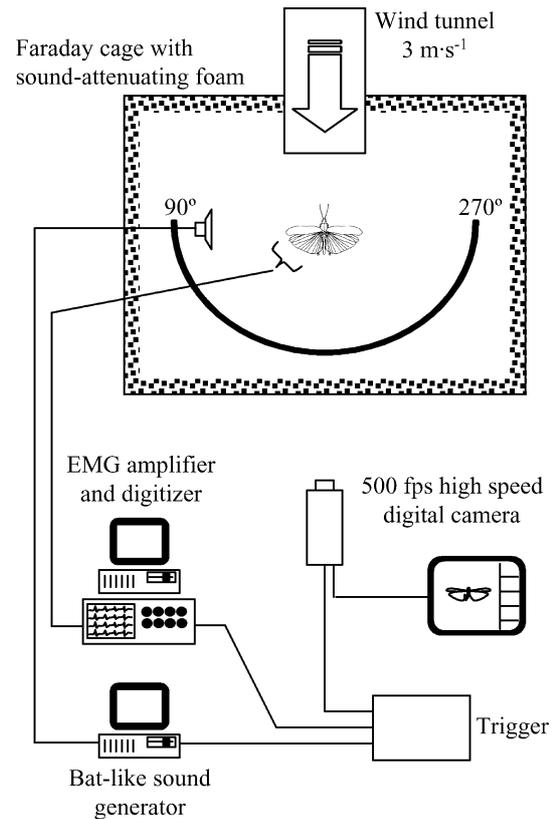
The observation of asymmetric wing depression in locusts avoiding collision with oncoming objects (Robertson and Reye 1992; Robertson and Johnson 1993) and when avoiding intense radiant heat sources (Robertson et al. 1996; Shoemaker and Robertson 1998) suggests that this may be a common strategy employed by locusts trying to evade hazards. Coordinated shifts in left and right forewing and hindwing depressor muscles have been recorded during thermal avoidance steering (Shoemaker and Robertson 1998) and were found to be correlated with asymmetric wing depression. However, this study did not employ high-speed cinematography and detailed kinematics of the stroke reversal could not be obtained. The purpose of this study was to determine the relationship between asymmetric depressor muscle activation and the kinematics of the stroke reversal. We have addressed this question with simultaneous recordings from direct depressor muscles and high-speed digital cinematography.

## Materials and methods

### Animals

Adult male and female *Locusta migratoria* L. aged 15–20 days post-imaginal moult were used in these experiments. All animals were reared under crowded conditions in a continuously breeding culture maintained at 30°C with 60% relative humidity under a 16 h:8 h light:dark cycle.

Animals were tested in a large (65-cm-deep, 125-cm-wide, 75-cm-tall) foam-lined Faraday cage (Fig. 2) with a circular wind tunnel (diameter 15 cm) providing an air stream of  $3 \text{ m s}^{-1}$  as measured with a hot-wire anemometer (TSI, model 67-7 with sensor model 1610-12). Animals were tethered dorsally by one of two methods. In some experiments (single muscle pair recordings), animals were attached directly to a rigid copper rod with a small drop of a mixture of beeswax and rosin powder placed on the pronotum. In other experiments (recordings of six muscle pairs), a small, aluminium saddle was fastened across the pronotum and secured with the same beeswax and rosin mixture as above. The aluminium saddle could be held in the jaws of an alligator clip and allowed easy removal of the animal from the experimental chamber between experiments. For all experiments, animals were suspended 10 cm in front of the wind tunnel and aligned with the wind stream. Adjust-



**Fig. 2** Diagram of the experimental apparatus. Locusts were tethered inside a large Faraday cage in front of a wind tunnel producing an air stream of  $3 \text{ m s}^{-1}$ . Sounds were presented from a speaker that could be positioned along an arc equidistant from the locust at any angle lateral and posterior to the locust. The Faraday cage was lined with contoured foam to minimize reflection of high-frequency sounds. Electromyogram (EMG) signals were amplified by a custom-built, 16-channel EMG amplifier and digitized at 25 kHz per channel. A high-speed digital camera (500 frames  $\text{s}^{-1}$ ) positioned directly behind the locust captured changes in body posture and wing kinematics. All recording equipment was synchronized by a common trigger allowing us to reconstruct the motor patterns underlying changes in wing kinematics resulting from the auditory stimulus

ments were aided by examining the locust with our high-speed camera (see below) for wing-stroke symmetry and limb alignment with the body. Animals were allowed to settle into a tucked flight posture (in which the forelegs were held up and beside the head capsule and the hindlegs were flexed and held against the abdomen) after which time stimuli were presented.

### Acoustic stimuli

Each stimulus consisted of a train of 30-kHz shaped pulses (10 pulses per train, 20-ms inter-pulse interval ( $= 30\text{-ms period or } 33.3 \text{ pulses s}^{-1}$ ), each pulse with a 1-ms exponential rise/fall time, 8-ms sustain time) presented from a 2-inch cone tweeter (Motorola, model KSN1078A). The speaker was positioned 20 cm from the locust with the centre of the speaker aligned

vertically with the locust tympanum. Sounds were always presented at least 2 min apart to avoid habituating the animal with repeated stimuli. Our sounds were digitally synthesized with an arbitrary waveform generator (model PCI-311; PC Instruments, Akron, Ohio, USA) controlled by a microcomputer with software supplied by the manufacturer (BenchTop Lite, v3.3 J). Sound presentation was initiated by way of a TTL trigger pulse generated by a Grass Instruments electronic stimulator (model S8; Grass Instruments, Quincy, Mass., USA) applied to the arbitrary waveform generator. Intensity was determined by matching pure tones of equivalent amplitude and frequency (dB peSPL; Stapells et al. 1982) to the stimulus pulses and measuring the SPL from a Brüel and Kjaer sound level meter (type 2610) with a Brüel and Kjaer 1/4-in. microphone (type 4135, without protective grid) positioned 20 cm in front of the speaker (where the locust tympanum was during experiments). The sound level meter was calibrated with a Brüel and Kjaer pistonphone (type 4228).

### Electromyography

Electrodes consisted of 80  $\mu\text{m}$  diameter (50- $\mu\text{m}$  core) copper wire insulated except at the tip. Unanaesthetized locusts were placed right side down in a large wax-filled Petri dish and secured with adhesive tape. Electrodes were then successively implanted into left M97, M98, M99, M127, M128 and M129 muscles (names after Snodgrass 1929) through small holes, made with a minuten pin, in the pterothoracic episterna and epimera. Vernacular names for the muscles are as follows: M97, M98 are the forewing first and second basalars respectively, M99 is the forewing subalar, M127, 128 are the hindwing first and second basalars respectively, M129 is the hindwing subalar. Electrodes were secured with a minimum of wax applied to the pleura and wires were guided and secured to the aluminium saddle at the pronotum. The locust was then turned left side down, re-secured with tape, and six more electrodes were implanted into the contralateral muscles with wires being similarly secured. Last, a common ground electrode was inserted through the distal region of the second abdominal tergite and secured with wax. The position of the ground electrode and wax did not obscure the ear and care was taken so that internal tympanal tracheal air sacs were not compromised. All 12 electrode wires and the ground wire were bundled together at the aluminium saddle and encased along their length to the recording amplifier in a small amount of flexible plastic adhesive (LePage 5F; Brampton, ON, Canada). After electrodes were implanted locusts were returned to their holding cages, with food, for 12–24 h for recovery before testing. The EMG electrodes had little effect on the ability of the locusts to flap their wings and generate flight and steering forces as we readily observed locusts performing climbing flights, turns, take-offs and landings while tethered loosely by the EMG wires alone.

Electrode signals were amplified with a custom built 16-channel differential amplifier (Ronald B. Harding) and digitized at 25 kHz per channel using hardware and software from Axon Instruments (Digidata 1200B with Axoscope v8.0; Axon instruments, Foster City, Calif., USA). The digitized traces included a record of the trigger pulse for presenting the stimulus. From the recordings, we measured the time of each muscle activation (as the time of the positive going peak of the waveform for the first spike in spike-bursts) and the number of spikes per burst for at least 15 cycles before and 15 cycles after the stimulus. Depending on depressor frequency, this window was between 1,200 and 1,600 ms.

Electrode position was confirmed after experiments by dissection. Animals were injected with a 1:4 mixture of 95% ethanol to glacial acetic acid and left at room temperature for 24–48 h. For each animal, the wings and legs were removed, the animal was decapitated and the abdomen was removed distal to the third abdominal segment. The thorax and remaining portions of the abdomen were then cut dorsally and ventrally along the midline and each hemisection was pinned in a water filled Petri dish with a cork bottom. The dorsal longitudinal and elevator muscles were then carefully removed to reveal the underlying depressor muscles. Each depressor muscle was then carefully teased apart until the tip of the electromyographic electrode was found. Results of electrode placement and inspection of the electromyographic recordings were used to determine which muscles were included in subsequent analyses.

### High-speed digital cinematography

Animals were filmed from behind using a Motion Scope 500-frame  $\text{s}^{-1}$  high-speed digital camera (model HR500; Optikon, Kitchener, ON, Canada). We used a 6-mm 1:1.4 TV lens and shuttered the camera at 1/2,500 s. We used a 250-W halogen lamp positioned above the camera and a 300-W halogen lamp positioned below the camera. Both lamps were directed onto the posterior of the locust from an angle of approximately 30° above and below, respectively. The light intensity at the locust was 135  $\text{W m}^{-2}$ . This light, which caused the temperature of the Faraday cage to rise above ambient room temperature, enabled us to complete our experiments at temperatures between 27 and 32°C. Blinded locusts (paint over eyes) and sighted locusts gave similar results (unpublished data).

To facilitate observation of the wings in the high-speed images, we applied marks to the wings with white correction fluid (Liquid Paper; Gillette Company, Boston, Mass., USA). We placed a dot at the tip of each of the four wings, a dot on the forewing where the costal vein meets the leading edge, and a narrow stripe to mark a wing chord on the forewing that began at the leading edge and ended where the second cubital vein meets the anal edge of the wing. These markings were placed on both upper and lower surfaces of the wing.

The high-speed camera was triggered with the same signal that initiated the sound presentations and therefore we were able to obtain simultaneous records of postural and wing kinematic changes with electromyographic recordings from flight muscles. After the camera was triggered, we copied a 1.5 s segment (750 frames) of the camera memory to videocassette at a rate of 1 frame  $s^{-1}$  for later analysis. The video tapes were then digitized using video digitizing hardware (All in Wonder 128; ATI Technologies, Thornhill, ON, Canada) and saved as AVI files. Custom software was then used to measure wing elevation angles, the time of stroke reversals, the onset of wing rotation at the stroke reversals (the timing of the wing flip), and abdomen position from the AVI files.

Our high-speed camera was placed directly behind the locust and imaged the locust along its posterior-anterior body axis. The optical axis of the camera was approximately orthogonal to the stroke plane of the beating wings. This simplified the task of extracting forewing and hindwing angles since they could be read directly from the images without requiring perspective correction (this would not have been the case had we viewed the locusts from an oblique angle). The beating wings are not confined to a flat stroke plane and we admit that this introduced some error into our measured wing angles, however, this error is small relative to the accuracy of our digitization method and was ignored. Wing angle measurements are  $\pm 5^\circ$  owing to the resolution of the camera and digitized AVI files.

The exact frame rate of our camera was determined by filming a light emitting diode that flashed at precisely measured intervals of time. The flash was generated by a Grass instruments stimulator (model S8; Grass Instruments, Quincy, Mass., USA) and the interval between flashes was measured with a digital storage oscilloscope (Gould, model DSO630). Frame-rate calculations were repeated several times using different intervals of time and with the camera cold (right after booting) and warm (on for at least 3 h). The average time between frames was  $2.0598 \pm 0.0003$  ms (mean  $\pm$  SD) and was not affected by camera temperature (operating time).

#### Data analysis and statistical treatment of the data

Spike times and elevation angles were used to calculate left-right motor and wing asymmetry respectively. These variables, and their calculations, are summarized in Table 1 and partially illustrated in Fig. 1. Calculations follow conventions established in Dawson et al. (1997). These variables are defined such that positive values always indicate an intended (fictive) turn to the right and negative values always indicate a fictive turn to the left. The data presented in Figs. 3, 7, 10, and 11 are from the same locust (L33R1) and illustrate the typical time course and cycle by cycle changes observed when a locust reacted to our stimulus.

Statistical treatment of the data followed procedures in Zar (1984) and Sokal and Rohlf (1981). Statistical

**Table 1** Definition and explanation of variables. These terms follow definitions in Dawson et al. (1997). See also illustrations in Fig. 1

Variable	Description (units)	Calculation <sup>a</sup> /notes <sup>b</sup>
DA	Depressor asymmetry (ms)	Calculated as the time of the left depolarization minus the time of the right depolarization. A positive value indicates the right muscle is activated before the left.
FWA-DS; HWA-DS	Fore- or hindwing asymmetry during the downstroke ( $^\circ$ )	The asymmetry in elevation angles <sup>a</sup> of the wings at one third of the downstroke. Calculated as the angle of the right wing minus the angle of the left wing. Positive values mean the right wing is more depressed than the left wing.
FW-SRA; HW-SRA	Fore- or hindwing stroke reversal asymmetry (ms)	Difference in time between the left and right stroke reversal. Calculated as the time of the left stroke reversal minus the time of the right stroke reversal. Positive values mean the right wing began the downstroke before the left wing.
FWA-SR; HWA-SR	Fore- or hindwing asymmetry at the stroke reversal ( $^\circ$ )	The asymmetry in elevation angles <sup>a</sup> of the wings at the moment of the stroke reversal (beginning of the downstroke of the respective wings). Calculated as the angle of the right wing minus the angle of the left wing. Positive values mean the right wing is more depressed than the left.
FW-PrA; HW-PrA	Fore- or hindwing pronation asymmetry (ms)	Refers to asymmetries in the time of the onset of pronation (the beginning of the wing flip). See Fig. 12. Calculated as the time of the left wing flip minus the time of the right wing flip. Positive values mean the right wing pronated (flipped) before the left.
ADA	Angular difference asymmetry ( $^\circ$ )	Refers to asymmetries in the "gap" between left and right fore- hindwing pairs. Angular difference is calculated by subtracting the elevation angle <sup>a</sup> of the forewing from the elevation angle of the hindwing. Asymmetries are calculated by subtracting the above difference from the left fore- hindwing pair from the difference calculated for the right fore- hindwing pair. A positive value indicates a smaller "gap" on the right side of the locust.

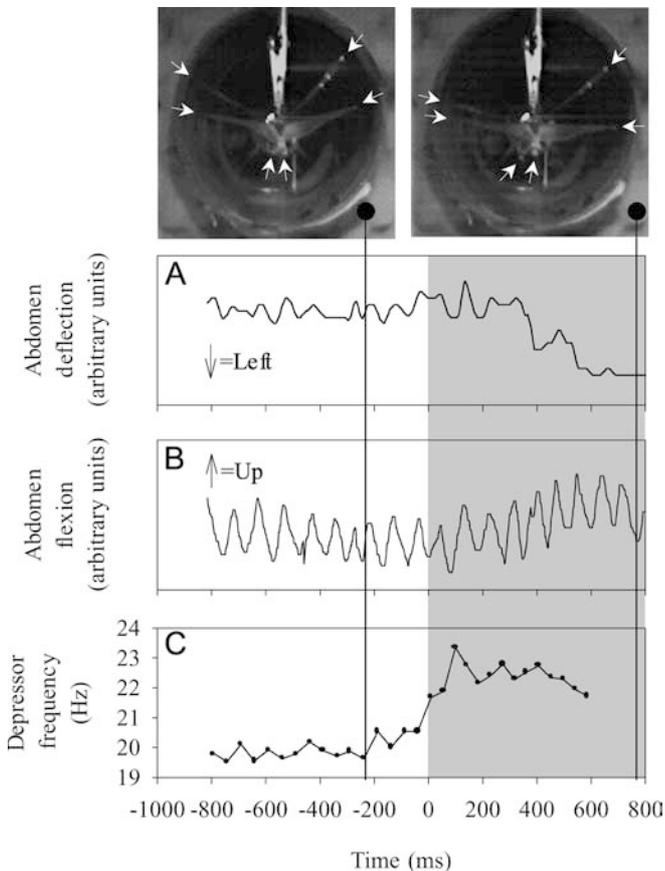
<sup>a</sup>Wing angles are measured with  $0^\circ$  directly above the locust

<sup>b</sup>The order of the terms in the calculations have been chosen such that positive results ( $^\circ$  or ms) reflect right "turns" and negative results reflect left "turns"

tests were performed using JMP v3.2.1 (SAS Institute). Significance was assumed when  $P \leq 0.05$ . Throughout this paper  $n$  refers to the number of animals tested.

## Results

Locusts began to fly as soon as they were placed in the wind stream. Most locusts required slight adjustment after securing the tether in front of the wind stream to achieve symmetrical wing beats and a proper flight posture although it was not possible to achieve perfect symmetry. When locusts were stimulated with sound, they reacted with abdomen and hindleg deflection, abdomen dorsiflexion, changes in wing kinematics, and increases in depressor muscle activation (wing beat) frequency (Fig. 3). From EMG recordings, we also saw shifts in depressor muscle timing coincident with changes in wing kinematics. A plot of wing elevation angle as



**Fig. 3A–C** Locusts responded to acoustic stimulation with lateral abdomen deflection, dorsiflexion, and an increase in depressor (wing beat) frequency. This locust (L33R1) responded with abdomen deflection to the left (A), abdominal dorsiflexion (B), and a 3 Hz increase in depressor frequency (C). Grey regions indicate the post-stimulus period. The images above are single frames from the high-speed camera taken at the times indicated. Arrows in the images indicate the position of the wing tips, the tip of the abdomen and the left hindleg (which deflected into the turn after stimulation). The low contrast in the images is a result of using a minimum of light during filming. Oscillations in B are due to the beating wings

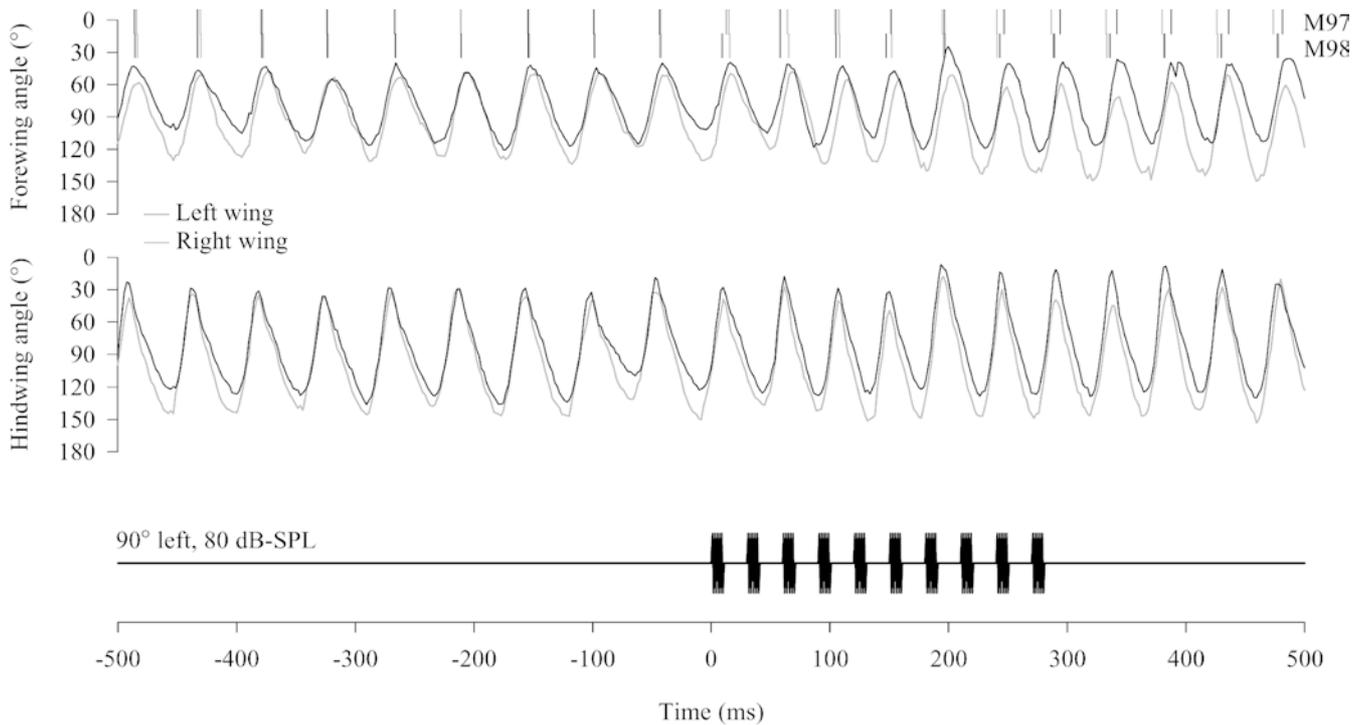
a function of time, for one locust (D17L1R1), shows asymmetric depression of the forewings and hindwings following stimulation (Fig. 4). Asymmetric wing depression was more pronounced in the forewings (particularly evident in the last six cycles of the forewings in the figure) than the hindwings. The right forewing was more depressed than the left forewing after stimulation and we interpret this as a fictive turn to the right. In this trace, stroke amplitude did not change substantially for either wing but the range of angles through which the wings swept changed. The change in forewing asymmetry was accompanied by asymmetries in the times of left and right depressor muscle activation in M97 and M98, the forewing first and second basalars, respectively.

In this study, we recorded responses to sounds presented from  $90^\circ$  left at 88 dB SPL from 24 locusts. From those 24, 19 showed fictive turns to the left, three showed fictive turns to the right, and for the remaining two locusts, turn direction could not be determined with certainty (Table 2). Turn direction was determined by looking at a number of behaviours: direction of abdomen deflection, forewing asymmetry measured at one-third downstroke, depressor asymmetry and angular difference asymmetry. In all but one locust there was consensus between all behaviours.

### Stimulus angle, intensity and latency to response

We determined latencies to key elements of the response described above (Fig. 5). From locusts that provided forewing first basalar (M97) records, we found that shifts in M97 depressor asymmetry (median = 80.6 ms, first and third quartiles = 46.0 ms, 104.8 ms, respectively,  $n = 15$ ) were coincident with shifts in forewing asymmetry measured during the downstroke (median = 82.4 ms, first and third quartiles = 45.3 ms, 134.9 ms,  $n = 15$ ), but substantially preceded abdominal dorsiflexion (median = 278.1 ms, first and third quartiles = 220.4 ms, 365.6 ms,  $n = 14$ ), and abdomen deflection (median = 316.2 ms, first and third quartiles = 226.6 ms, 406.8 ms,  $n = 14$ ).

We tested the effect of stimulus intensity ( $n = 6$ ) and direction ( $n = 13$ ) on the magnitude and direction of forewing M97 depressor asymmetry (these locusts are different from those used for motor pattern recordings). The magnitude of depressor shifts increased with increasing intensity of the stimulus (Fig. 6A). Depressor asymmetry shifts, without regard to direction, elicited with 60- and 65-dB SPL sounds were  $3.1 \pm 1.1$  ms (mean  $\pm$  SEM) and were significantly different than shifts elicited with 70-dB SPL sounds and higher which were  $7.5 \pm 1.1$  ms (ANOVA of treatments by subjects,  $F = 5.75$ ,  $df = 5, 5$ ,  $P = 0.006$ ; means compared post-hoc with multiple Student's  $t$ -tests,  $P < 0.05$ ). Depressor frequency (Fig. 6B) increased for all locusts tested by  $2.0 \pm 0.4$  Hz from  $17.1 \pm 0.7$  Hz after stimulation but the magnitude of the increase was not dependent on



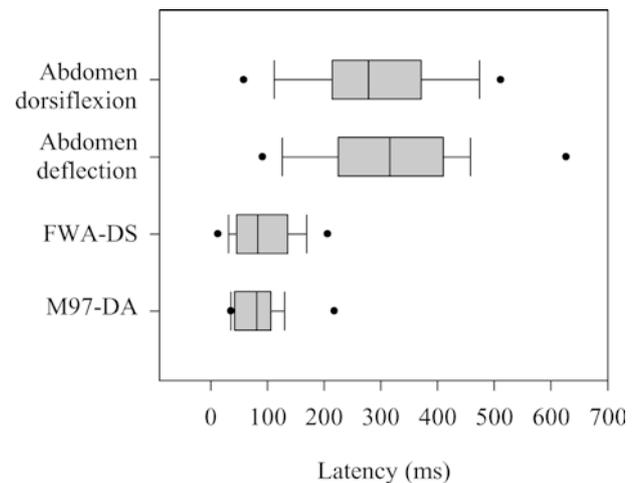
**Fig. 4** Plot of forewing and hindwing angles with accompanying raster plot of left and right M97 and M98 from one locust (D17L1R1). The left and right forewing stroke angles become asymmetric during the downstroke approximately 200 ms after stimulus onset. This is coincident with asymmetric activation of the forewing first basalar, M97, and the forewing second basalar, M98. Asymmetries in hindwing angles during the downstroke are not as pronounced as for the forewings. Note the increase in wing beat frequency after the stimulus. *Black traces* are the left wing and left muscles, *grey traces* are the right wing and right muscles

stimulus intensity (ANOVA of treatments by subjects,  $F=1.02$ ,  $df=5,5$ ,  $P=0.431$ ). The magnitude of M97 depressor shifts, elicited with an 80-dB SPL stimulus, did not depend on stimulus angle (Fig. 6C) (ANOVA of treatments by subjects,  $F=0.51$ ,  $df=12,3$   $P=0.683$ ) nor was the number of animals that showed left or right M97 depressor shifts (Fig. 6D) (contingency table  $G$ -test,  $G=2.668$ ,  $df=3$ ,  $P=0.431$ ).

#### Motor pattern

We implanted EMG electrodes into the forewing first and second basalars, M97 and M98 respectively, and the subalar, M99, and the hindwing first and second basalars, M127 and M128, respectively, and the subalar, M129. From the 24 locusts stimulated with sound, we obtained successful EMG recordings from 15 M97 left-right pairs, 6 M98 pairs, 17 M99 pairs, 21 M127 pairs, 15 M128 pairs, and 22 M129 pairs. Only two animals yielded data for all six muscle pairs simultaneously. We typically obtained data from at least four muscle pairs for a given locust. A recording from one of the two locusts in which all 12 direct downstroke muscles were successfully penetrated is presented in Fig. 7.

Prior to stimulus presentation, we saw depolarizations with each wing stroke cycle in nearly all depressor muscles. In certain animals, spiking was absent for many cycles in forewing M99, and to a lesser extent M98, and hindwing M128. Spiking in these muscles would resume immediately after the stimulus was presented. This is seen in the EMG recorded from the locust presented in



**Fig. 5** Latencies of components of the acoustic startle response (ASR) elicited with 30-kHz pulsed sounds presented at 88 dB SPL from 90° left. The first evidence of steering occurred as shifts in depressor asymmetry (and spikes per burst) (median = 80.6 ms, first and third quartiles = 46.0 ms, 104.8 ms, respectively,  $n=15$ ) and asymmetric forewing depression (median = 82.4 ms, first and third quartiles = 45.3 ms, 134.9 ms,  $n=15$ ), which were followed by abdomen dorsiflexion (median = 278.1 ms, first and third quartiles = 220.4 ms, 365.6 ms,  $n=14$ ) and abdomen deflection (median = 316.2 ms, first and third quartiles = 226.6 ms, 406.8 ms,  $n=14$ )

Locust	Abdomen Deflection	Abdomen Flexion	FWA-DS	DA <sup>a</sup>	ADA
9	Left	Up	-	-	-
10	Left	Up	-	-	-
11	Left	Up	-	-	-
12	N/C <sup>b</sup>	Up	N/C	N/C	N/C
13	Left	Up	-	-	-
15	Right	Up	+	+	+
16	Left	Up	N/C	-	N/C
17	Left	Up	-	-	-
18	Left	Down	-	-	-
19	Left	Up	-	-	-
20	N/C	N/C	-	-	-
21	N/C	Up	-	-	-
22	Right	Up	-	+	-
24	N/C	Up	-	-	-
25	Left	Up	-	-	-
26	Left	Up	-	-	-
27	Left	Up	-	-	-
28	Left	Up	-	-	-
29	Right	Up	+	N/C	+
30	Left	Up	-	-	-
31	N/C	Up	+	+	N/C
32	Left	Up	-	-	-
33	Left	Up	-	-	-
34	Left	Up	-	-	-

<sup>a</sup>DA is measured from forewing depressors, usually M97; empty cells indicate no forewing depressor activity was recorded for that locust.

<sup>b</sup>N/C refers to situations where we could not discern a clear change in the post-stimulus response from the pre-stimulus condition.

**Table 2** Summary of correspondence between direction of abdomen deflection, sign of forewing asymmetry during the downstroke, sign of forewing depressor asymmetry, and sign of angular difference asymmetry. All locusts were stimulated with 30-kHz pulsed sounds (see text) at 88 dB SPL from 90° to the left. Of 24 locusts included in the data set, 19 locusts fictively turned left, three fictively turned right (*light grey*), and two could not be determined (*dark grey*). The latter two were included in the data set because other aspects of the motor pattern, (e.g. abdomen flexion, increases in depressor frequency, changes in depressor spiking), suggested that they reacted to the stimulus. Only one locust (22), produced contradictory information (abdomen deflection to the right with positive depressor asymmetry but negative forewing asymmetry during the downstroke and negative angular difference asymmetry). (+) indicates a positive shift in the variable, (-) indicates a negative shift in the variable (see Table 1 for explanation of variables)

Fig. 7 for M98, M99 and M128. For all locusts, following presentation of the stimulus, several changes occurred in the motor pattern. Muscles that were not being rhythmically depolarized began to fire with every wing beat cycle. There was an increase in the number of depolarizations seen with each cycle (hereafter referred to as spikes per burst). The frequency of depressor muscle activation increased and the timing of depolarizations advanced in the cycle relative to the hindwing stroke reversal. Lastly, depolarization of contralateral muscle pairs became asymmetric (more asymmetric) (boxed region of Fig. 7).

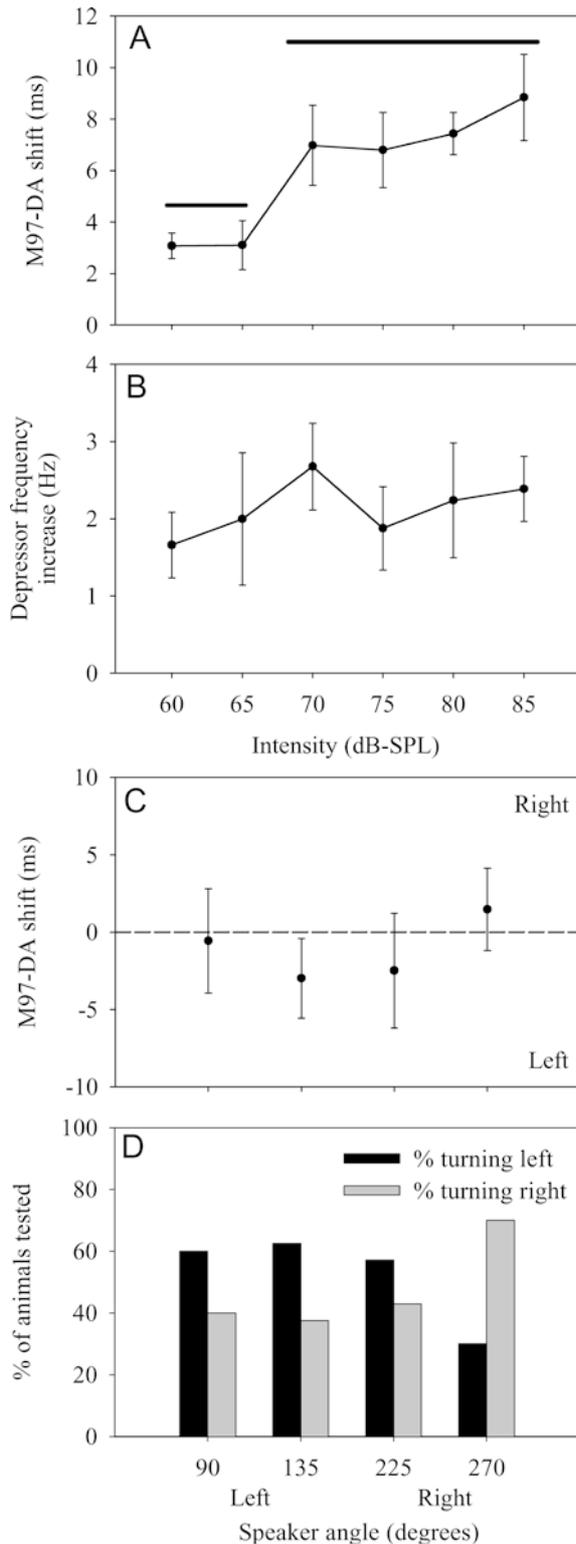
#### Spikes per burst

Before a stimulus was applied, depolarizations of the muscles occurred as singlet or doublet waveforms (spikes). After stimulation, we observed an increase in

the number of spikes per burst in all depressor muscles on both the left and right side of the locust. The increase occurred irrespective of the direction of the fictive turn. Close examination of locusts that fictively turned to the left confirms this (Fig. 8). Significant increases were found in all depressor muscles (paired t-tests,  $P < 0.05$ ) on both the left and right side of the locust. The increase in spikes per burst was seen even when abdomen deflection and forewing asymmetry were not apparent (e.g. locust 12, Table 2).

#### Shifts relative to the hindwing stroke reversal

From the 19 animals that fictively turned left, we calculated the time of activation of each muscle (taken as the first spike in a burst) relative to the hindwing stroke reversal (Fig. 9). We chose the hindwing stroke reversal as a reference of cycle time because asymmetries in the hindwing stroke reversals are not as pronounced as they are for the forewings during steering. The hindwing stroke reversal time was calculated by taking the average of the left and right wing stroke reversal times. There was considerable variability in activation times of all the muscles in the cycle relative to the hindwing stroke reversal time before the stimulus was presented and depolarizations of contralateral muscle pairs often did not fire simultaneously as might be expected for straight flight. From the medians of the spike times, hindwing muscles depolarized before the forewing muscles. Both before and after the stimulus, the first basalars (M97, M127) and subalars (M99, M129) were depolarized before the second



basalars (M98, M128) for the forewings and hindwings. Following stimulation, this pattern persisted but all muscles shifted and fired earlier in the stroke. Finally, following stimulation, the left muscles fired earlier than the right muscles except M127 in which the right muscle began to depolarize before left.



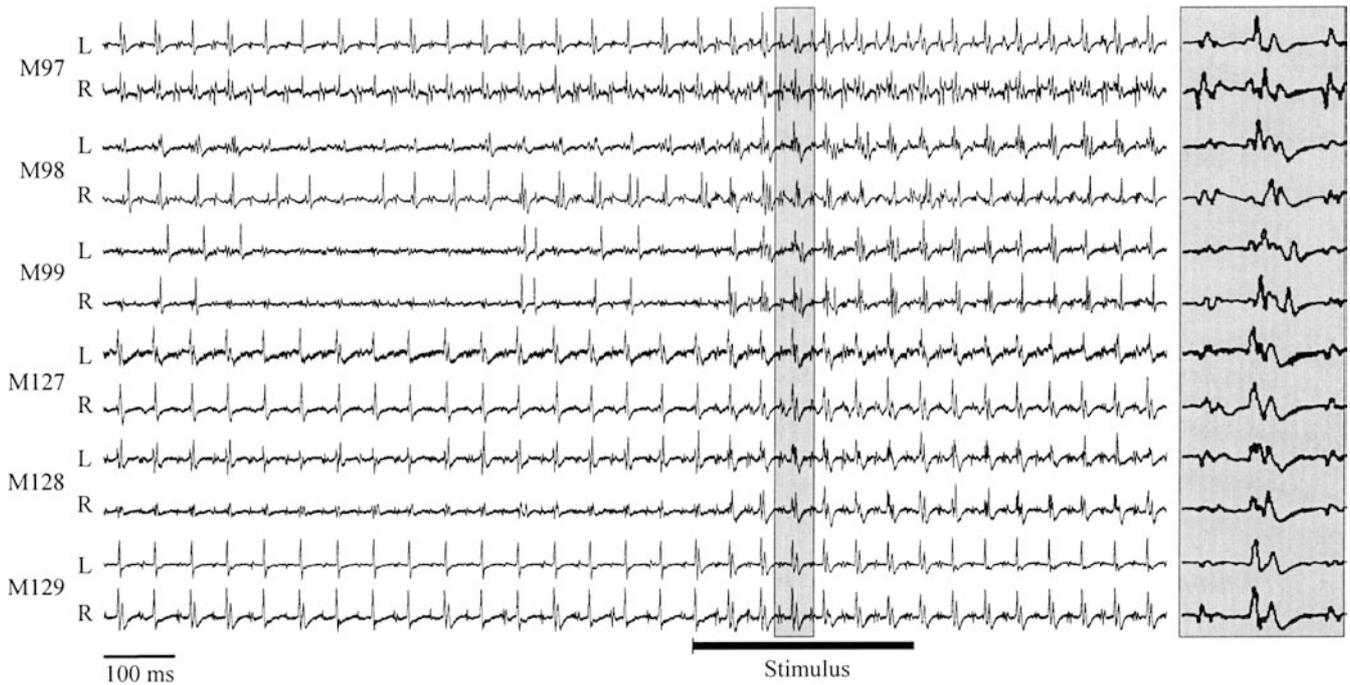
**Fig. 6 A** Forewing first basalar (M97) depressor asymmetry shifts (post-stimulus peak minus pre-stimulus median value, calculated irrespective of direction) increased with increasing stimulus intensity. Data are mean  $\pm$  SE ( $n=6$  for all intensities except 65 and 70 dB SPL where  $n=5$ ). Horizontal bars indicate significant differences. **B** Depressor frequency increased approximately 2 Hz after stimulation but the increase was not dependent on stimulus intensity (data from the same locusts as **A**; sample sizes as in **A**). **C** M97 depressor asymmetry shifts, a measure of steering direction, were not dependent on the position of the speaker when placed at different angles beside and behind the locust.  $0^\circ$  is directly in front of the locust,  $90^\circ$  is left of the locust. Values are mean  $\pm$  SE ( $n=10$  at  $90^\circ$ ,  $n=8$  at  $135^\circ$ ,  $n=7$  at  $225^\circ$ ,  $n=10$  at  $270^\circ$ ). **D** The number of locusts showing positive or negative depressor asymmetries (i.e. making fictive turns to the right or left, respectively) was not contingent on speaker position (data from the same locusts in **C**; sample sizes as in **C**)

#### Shifts of fore- and hindwing contralateral muscle pairs

Asymmetries in contralateral depressor muscle activations were abrupt with the onset of the stimulus and often appeared within the first three post-stimulus wing beat cycles. Depressor asymmetries in all forewing muscles, M97, M98, and M99, shifted together whereas hindwing M127 and M128 depressor asymmetry shifted opposite to M129. The time-course of shifts in forewing and hindwing muscles for one locust, L33R1, is presented in Fig. 10A, F. Some locusts showed an initial shift in one direction followed by a prolonged shift in the opposite direction. From the 19 locusts that turned left, we calculated the magnitude of depressor shifts by taking the average (across locusts) of the differences between median post-stimulus and median pre-stimulus depressor asymmetries (for each locust) calculated on a cycle by cycle basis. The magnitude of shifts in forewing M97 depressor asymmetry was smallest at  $6.62 \pm 0.54$  ms (mean  $\pm$  SEM) in comparison to the other forewing muscles and was the least variable. M98 depressor asymmetry shifts were  $9.26 \pm 2.08$  ms and shifts in M99 depressor asymmetry were  $12.05 \pm 2.05$  ms. Shifts in hindwing depressor asymmetries were smaller than shifts in forewing depressor asymmetries. M127 shifts were  $3.79 \pm 0.52$  ms, shifts in M128 were  $5.46 \pm 0.91$  ms and shifts in M129 were  $2.15 \pm 0.54$  ms.

#### Correlation of motor activity and wing kinematics

Asymmetries in all forewing kinematic variables shifted in time with, and in the same direction as, asymmetries in the forewing muscles at the onset of the stimulus (Fig. 10B–E). Corresponding shifts in hindwing kinematic variables either did not occur or were not as pronounced as those in the forewings (Fig. 10G–J). When hindwing shifts were present they occurred in the opposite direction than those of the forewings (e.g. hindwing asymmetry measured at one-third downstroke, Fig. 10G compared with Fig. 10B). Shifts in angular difference asymmetry are plotted in Fig. 10 K.



**Fig. 7** EMG recordings from one locust (L33R1) from 12 direct downstroke muscles, 6 in the forewings and 6 in the hindwings, 3 per side for each wing. Before the stimulus M98 and M99 fire intermittently but when firing the spikes are in time with the wing cycle. After the stimulus, all muscles are active and there is an increase in number of depolarizations per cycle (spikes per burst) for all of the muscles that last for several cycles post-stimulus. *Grey area* is presented on an expanded time scale at the right to show asymmetries in contralateral depressor muscle pairs. Forewing depressor muscles typically show larger shifts than hindwing depressors. The stimulus is a 30-kHz high-frequency, pulsed, sound presented from 90° left of the locust at 88 dB SPL (additional details in text)

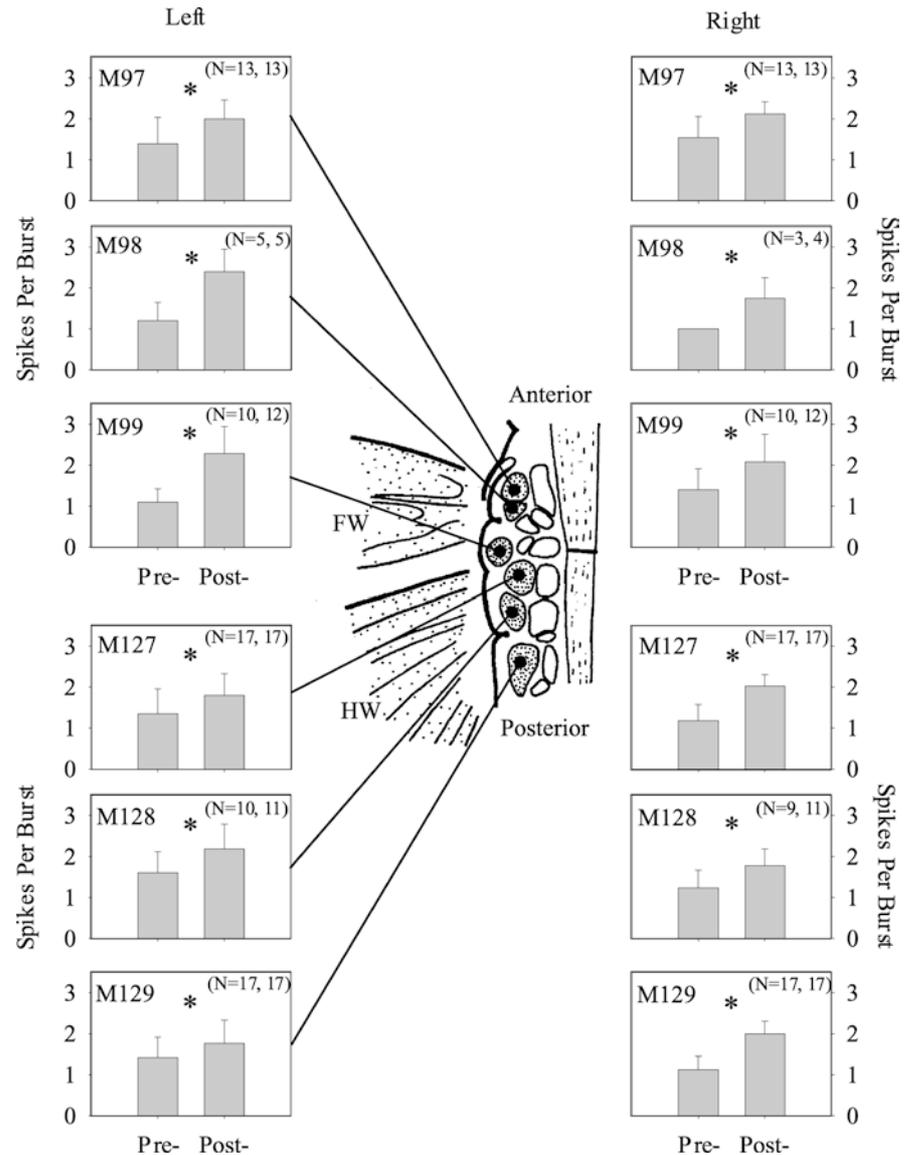
Due to high cycle to cycle variability in depressor activity and wing kinematic measurements, we performed pairwise correlations between motor and kinematic variables for each locust individually on a cycle-to-cycle basis. This is in contrast to a method that would take averages of shifts over a number of cycles for each locust and then perform correlations on the average shifts across all locusts. We feel the former method gives a more accurate impression of steering because the cycle-to-cycle variability, which may be an important aspect of motor control, is preserved in the analysis. A scatter plot matrix of all pairwise combinations of depressor muscle activity and wing kinematic measurements is shown for the forewings (Fig. 11) for one locust, L33R1. Because we generated correlation matrices for each of the locusts, we present one matrix to illustrate the cycle-to-cycle shifts and summarize the results for all 24 locusts in Table 3. The strongest correlations were found when forewing M97 depressor asymmetry was correlated with the forewing kinematic variables. Strong correlations were also found for both motor and kinematic variables correlated with angular difference asymmetry. Correlations of hindwing motor and kinematic variables were

weaker, and frequently non-significant, owing to the fact that changes in the hindwings are smaller than those of the forewings.

Correlations were performed for all combinations of depressor muscle asymmetries and wing kinematic measurements for each of the 24 locusts tested (regardless of steering direction and magnitude of response). For each variable pair, we then tallied the number of significant positive and significant negative correlations. From only the significant correlations, we determined which variables (muscle shifts and wing kinematic shifts) tended to shift together (positive correlations) or oppositely (negative correlations). The data from all 24 locusts is summarized in Table 3 including the number of locusts that yielded data for each pairwise combination and the number of non-significant correlations.

When examining this table it is important to look at the relative numbers of positive and negative correlations and not the number of significant correlations relative to non-significant correlations. This is because non-significant correlations could have resulted from two factors: that there was no biological relationship (i.e. no shift in the variables), or that the steering response was small (i.e. that the shift in one or both variables was small). For example, the correlation between forewing asymmetry at one third downstroke and M99 depressor shifts was calculated for 16 animals. Of the 16 correlations, 7 were positive, none were negative and 9 were non significant. Here, it is wrong to conclude that M99 does not shift with forewing asymmetry because there were more non-significant correlations than significant correlations, or indeed that less than half the correlations were significant. It is important to remember that some of the non-significant correlations resulted from small shifts that were not statistically detectable.

**Fig. 8** The number of depolarizations per cycle (spikes per burst) increased after stimulation on both the left and right sides in locusts. Data above are for locusts fictively turning left only. For each locust, the median number of spikes per burst was calculated for the pre-stimulus and post-stimulus period. The median pre-stimulus value was measured from the 15 cycles preceding the stimulus and the post-stimulus value was measured from the first 10 cycles after the stimulus began. The mean  $\pm$  SD of the medians are plotted with sample sizes for the pre- and post stimulus, respectively, indicated in *parentheses*. Sample sizes for pre- and post-stimulus are different for certain muscles because these muscles were not active before the stimulus was applied. *Asterisks* indicate significant differences (paired *t*-tests,  $P < 0.05$ ). The diagram in the centre shows the relative location of the forewing and hindwing direct downstroke muscles within the left side of the pterothorax of the locust (dorsal view) (modified from Möhl and Zarnack 1977)



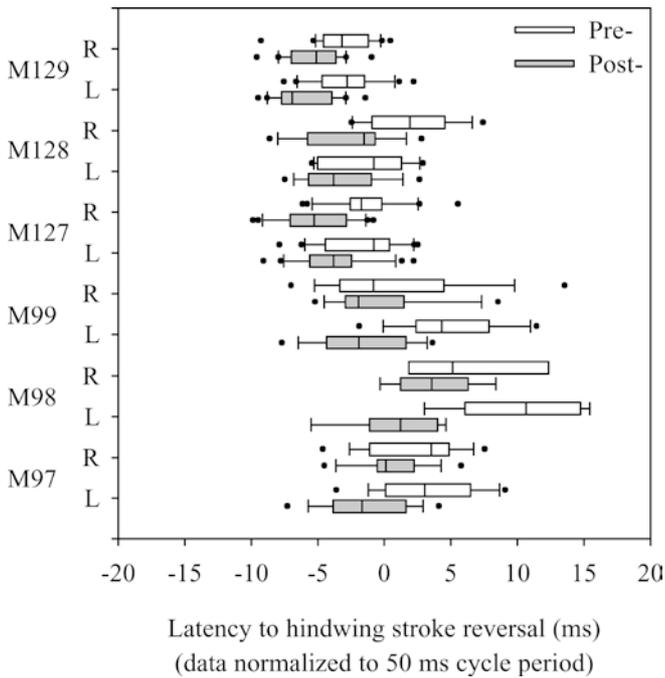
Therefore, to be conservative, we decided not to try to distinguish between non-significant correlations resulting from small shifts (which may be biologically significant) and non-significant correlations resulting from a lack of biological relationship. Thus, in our example, the meaningful interpretation comes in noting that seven out of seven significant correlations were positive (i.e. that forewing asymmetry and M99 depressor asymmetry shift in the same direction).

The data in Table 3 can be summarized as follows. With regard to the forewing motor and kinematic correlations, depressor asymmetries in forewing muscles, M97, M98, and M99 were all positively correlated with each other meaning they shifted together, in the same direction. This is to say, an increase in M97-DA was accompanied by an increase in M98-DA and M99-DA. All forewing kinematic variables shifted with forewing depressor asymmetries. The strongest correlations were between M97-DA and the forewing kinematic changes. Also of note are the correlations between forewing

asymmetry at the stroke reversal (FWA-SR) and forewing stroke reversal asymmetry (FWA-SRA). Here no clear pattern emerged as most of the correlations were non-significant.

With regard to the hindwing motor and kinematic correlations, hindwing depressor asymmetries in M127 shifted with depressor asymmetries in M128 but were opposite in direction to shifts in M129 depressor asymmetry. Correlations of depressor asymmetries in M129 and hindwing asymmetry during the downstroke (HWA-DS) were also primarily negative. We note that motor and wing kinematic changes were small in magnitude and this has resulted in relatively few significant correlations. The notable exception to this, however, are the correlations between hindwing stroke reversal asymmetry and hindwing asymmetry at the stroke reversal where 16 of 16 significant correlations were negative.

Considering forewing and hindwing motor correlations, forewing M97, M98 and M99 depressor asymmetries predominantly produce negative correlations



**Fig. 9** Spike times of the forewing and hindwing direct downstroke muscles calculated relative to the time of the hindwing stroke reversal (normalized to a 50-ms wing beat period). Data are taken from locusts turning to the left only. All muscles fired earlier in the stroke when presented with a 30-kHz pulsed sound from 90° to the left at 88 dB SPL. For each muscle, we calculated the latency of each depolarization (on a cycle by cycle basis) to the hindwing stroke reversal (median of left and right stroke reversal). These latencies were then normalized to a 50 ms cycle period. The median of all the pre-stimulus latencies was then calculated as was the median of all the post-stimulus latencies. These values were determined for all 19 locusts tested that turned left (as determined by consensus of direction of abdomen deflection, wing asymmetry, and depressor asymmetry) and were plotted as separate box plots for the pre-stimulus (*white boxes*) and post-stimulus (*shaded boxes*) period. We chose the hindwing stroke reversal because the hindwings show smaller shifts during steering than the forewings and therefore represented a stable marker of the time of each wing beat cycle. *Boxplots* show the first and third quartiles with the median indicated by the *line* within the box. *Whiskers* show 10th and 90th percentiles and *dots* show data outside of the aforementioned percentiles

with hindwing M127 and M128 depressor asymmetries (but not M129 depressor asymmetries). Therefore, shifts in asymmetries of the forewing muscles are opposite to those of the hindwing first and second basalars. The forewing and hindwing kinematic correlations also reflect the pattern of shifts seen in the muscles. Note forewing asymmetry during the downstroke is negatively correlated with hindwing asymmetry during the downstroke (12 of 13 correlations are significant, negative correlations).

Lastly, when considering the interactions between forewings and hindwings, the correlation matrix shows that shifts in the forewing depressor asymmetries and kinematic asymmetries are primarily positively correlated with angular difference asymmetry, a measure of the shift in the relative gaps between the contralateral forewings and hindwings. The opposite is observed in

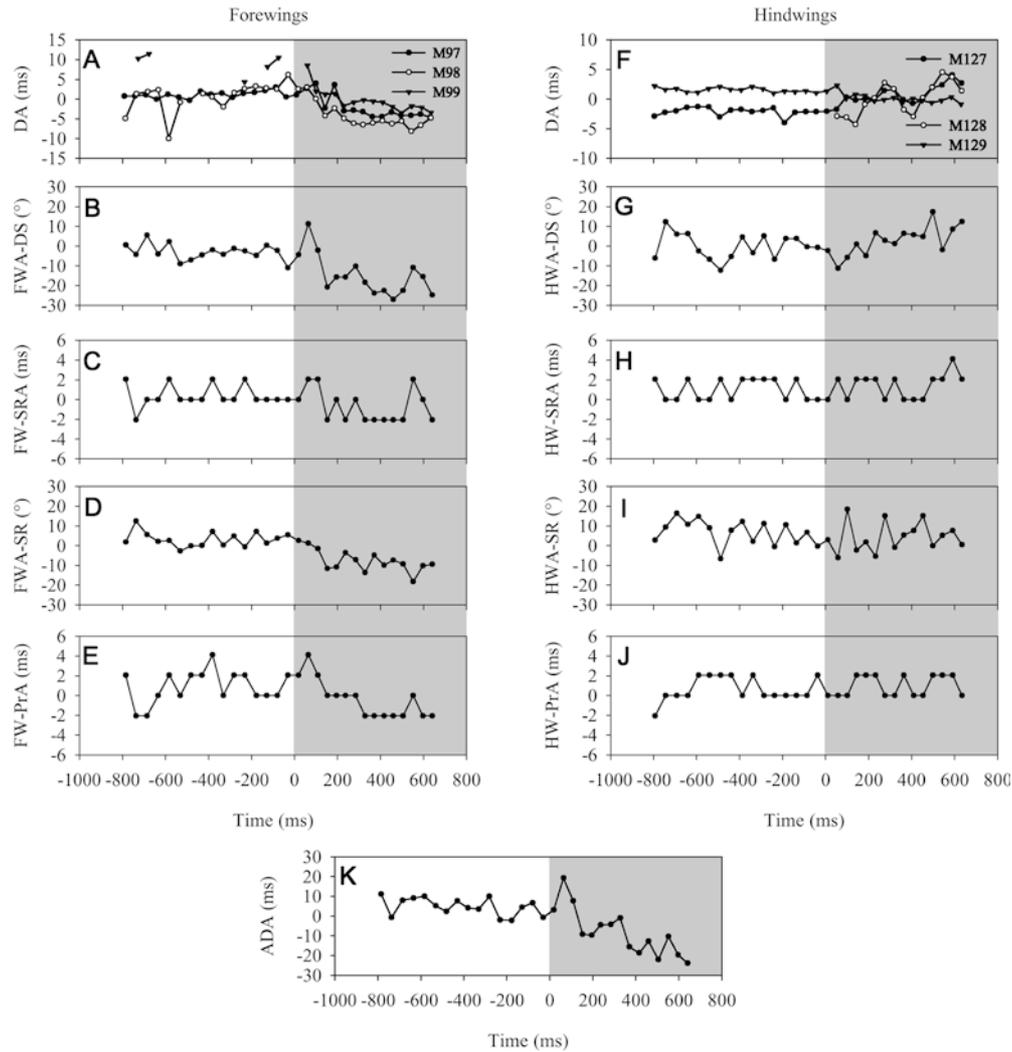
hindwing depressor and kinematic asymmetries correlated with angular difference asymmetry except for hindwing M129 depressor asymmetry.

#### Timing of the (dorsal) wing flip

At the end of each half-stroke, the wing must rotate to establish an appropriate angle of attack for translation. The dorsal wing flip is the rotation of the wing (pronation) at the end of the upstroke to establish an appropriate angle of attack for the downstroke (Fig. 12A). Here, it is important that we stress that we have not measured the angle of attack of the left and right wings *during the downstroke*, but rather the timing of the onset of the rotation, at the end of the upstroke, that leads to the establishment of angle of attack. Limitations in our high-speed digital images meant that we could not obtain accurate, quantitative measures of this parameter; however, we did obtain approximate measures, for illustrative purposes, for the locust (L13R1) presented in Fig. 12A. This figure shows the time-course of changes in wing stroke angles and approximate angle of attack with rotational velocity. Although quantitative measures of angle of attack were not possible for the locusts in this study, we note the wing on the inside of the turn path, the left wing, was more pronated than the right wing during the downstroke.

The time course of changes in the latency of wing pronation (time between the onset of wing rotation and the stroke reversal) for the left and right wings of one locust (L34R1) is shown in Fig. 12B. Changes in latency were coincident with the development of forewing asymmetry measured during the downstroke. Before the stimulus was applied, at the end of the upstroke, all locusts began rotation of the right wing before the left (latency of right wing pronation to stroke reversal =  $12.14 \pm 2.44$  ms (mean  $\pm$  SD); latency of left wing pronation to stroke reversal =  $6.40 \pm 2.05$  ms). Following stimulation, the right wing continued to begin pronation before the left (latency of right wing pronation to stroke reversal =  $12.14 \pm 1.93$  ms; latency of left wing pronation to stroke reversal =  $5.96 \pm 1.36$  ms). Although the mean latencies may have not changed appreciably, the pattern of shifts in latencies were indicative of a strategy to decrease the latency of wing rotation on the left side and increase the latency of wing rotation on the right side.

Of the 19 locusts that turned left, 14 (74%) showed a change in latency for either left or right or both wings together. Of five locusts that changed the latency of pronation of the left wing, four (80%) decreased the latency of pronation to the stroke reversal (i.e. shifted the beginning of pronation closer to the time of the stroke reversal) (magnitude of shift ranges 2.06–4.12 ms) while seven (58%) of the 12 locusts that changed the latency of pronation to the right wing stroke reversal increased that latency (magnitude of shift ranges 1.02–6.18 ms).



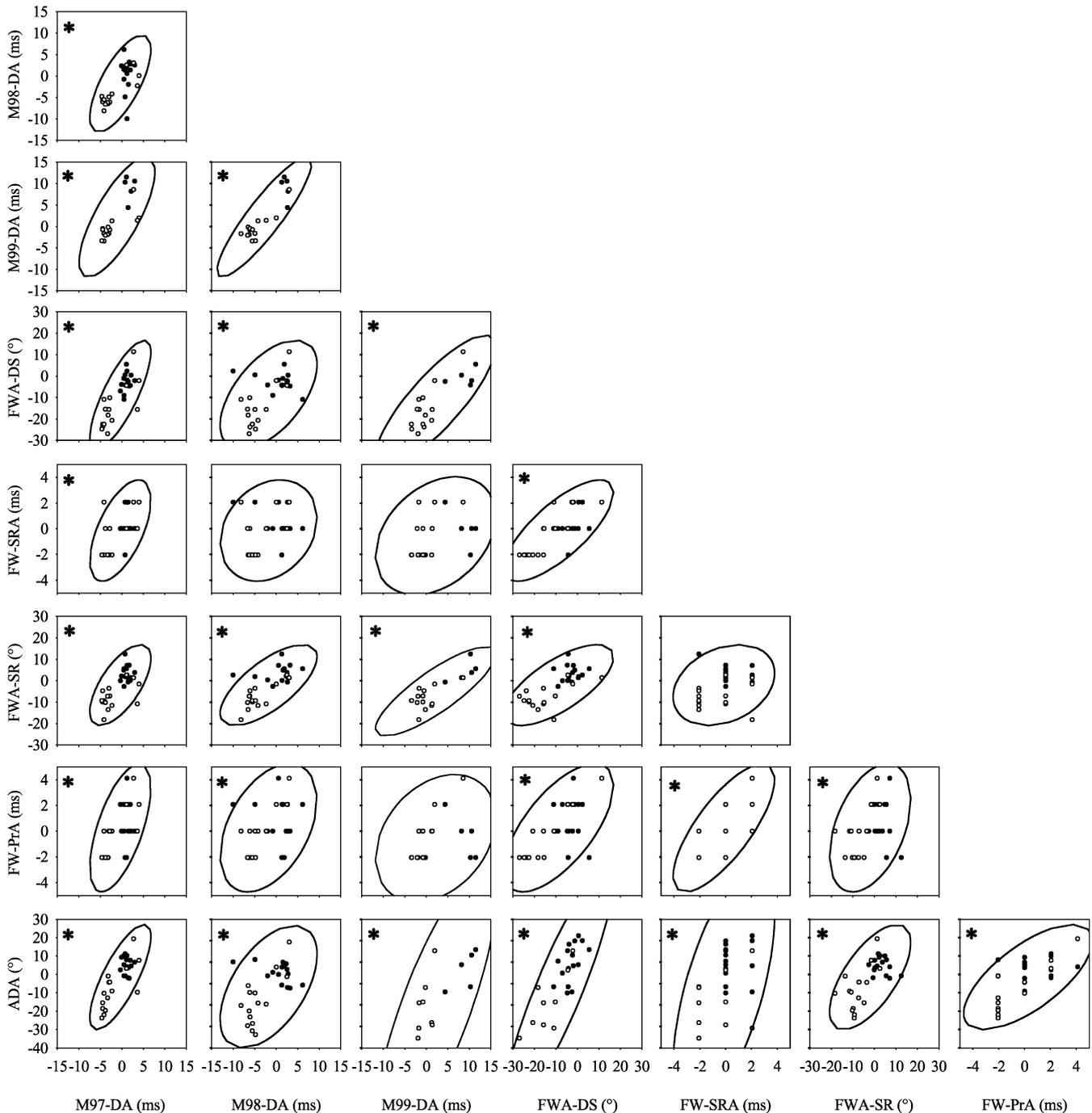
**Fig. 10A–J** Time-course of forewing and hindwing motor pattern and kinematic changes for one locust (L33R1) following stimulation with a 30-kHz pulsed sound at 88 dB SPL from 90° to the left (*grey region*). **A** Depressor asymmetries for all three forewing muscles shift to the left (negative). The onset of depressor shifts are coincident with shifts in all the forewing kinematic variables measured: **B** forewing asymmetry measured at one-third of the downstroke, **C** forewing stroke reversal asymmetry, **D** forewing asymmetry at the stroke reversal, and **E** forewing pronation asymmetry. Forewing asymmetry measured at one-third of the downstroke showed an initial shift to the right (positive) followed by a large and prolonged shift to the left. This is also seen in **C**, **E** and **K**. **F** Hindwing depressor asymmetries are less pronounced than those of the forewings. M127 and M128 depressor muscles shift to the right (positive) while M129 shifts to the left (negative). **G**, **H**, **I**, **J** The coincident changes in hindwing kinematic variables is less obvious; hindwing shifts are small in comparison to forewing shifts. **K** Angular difference asymmetry; a measure of the relative separation between forewings and hindwings on the left and right shows that a greater fore-hindwing gap forms on right side of the locust (i.e. on the outside of the turn path). Because the hindwing shifts are small, this gap is mostly created by asymmetric forewing depression

Forewing pronation asymmetry (FW-PrA), a measure of the relative timing of left and right wing pronation, was strongly and positively correlated with M97 depressor asymmetry (11 of 12 significant correlations

were positive) and forewing asymmetry measured during the downstroke (14 of 14 significant correlations were positive) (Table 3).

## Discussion

Behavioural observations of locusts in free flight have shown that locusts react to high-frequency pulsed sounds with a change in flight path away from the source of the sound (Dawson et al. 2004). Dawson et al. (1997), using the same sound patterns, showed that tethered locusts react with asymmetric depression of the forewings and hindwings during the downstroke and argued that this may be an aerodynamically favourable configuration, especially when combined with other wing kinematic changes (asymmetrical wing pronation) and postural adjustments (abdomen and hindleg deflection). Dawson et al. (1997) argued that the motor strategy of a locust in this situation may be to adjust the timing of the stroke reversal to establish asymmetric wing depression (Fig. 1) to tip the direction of net lift production by the left and right wing couple. An alternate strategy



**Fig. 11** Scattergrams of all pairwise correlations for one animal (L33R1) of depressor asymmetry and kinematic changes for the forewings. Each X-Y pair in a scatterplot is from a single wingstroke cycle. *Black dots* are from cycles before the stimulus, *grey dots* are from cycles after the stimulus (note in some scatterplots the post-stimulus data is plotted on top of the pre-stimulus data). *Ellipses* are 95% density regions of the correlation (enclosing 95% of the data within the scatter plot). The greater the ratio of the major to minor axis lengths, the stronger the correlation. *Asterisks* indicate significant correlations (Pearson product moment,  $P < 0.05$ )

suggested was that locusts may adjust the wing stroke angles so as to minimize or maximize the relative gap between forewings and hindwings on the inside and

outside of the turn path, respectively. In this paper we have examined tethered locusts reacting to high-frequency sounds with simultaneous high-speed cinematography and multi-channel electromyography to determine if contralateral shifts in the timing of depressor activation correlate with the kinematics of the stroke reversal and the establishment of asymmetric wing depression. We present data that shows the kinematics of the stroke reversal are correlated with asymmetric wing depression and asymmetric depressor muscle timing. Given that the aerodynamic interactions between forewings and hindwings are largely unknown,

our data does not exclude the possibility that the motor patterns we recorded are important for establishing a favourable fore-hindwing kinematic configuration.

The reactions of the locusts in this study are consistent with observations from Robert (1989) and Dawson et al. (1997). In response to stimulation with sound, we observed abdomen deflection and dorsiflexion, increases in depressor (wing beat) frequency, asymmetric wing depression in the forewings and hindwings, and changes in depressor muscle activity. Changes in abdomen deflection (Camhi 1970) are correlated with steering (both intentional and corrective) and their role, with abdomen dorsiflexion and changes in depressor (wing beat) frequency, have been addressed in an earlier paper by Dawson et al. (1997). We therefore limit our discussion to the topic of forewing asymmetry and its underlying motor control as well as the possible significance of our observations of the timing of the dorsal wing flip.

#### Stimulus angle, intensity, and latency to response

Reactions in our locusts (Fig. 5), first evident as shifts in depressor asymmetry, occurred with a median latency of 80.6 ms which corresponds to as few as two wing beat cycles after stimulus onset. The magnitude of depressor shifts was intensity dependent and seemed to show a discrete (step-wise) increase for intensities 70 dB SPL and greater (Fig. 6A). Robert (1989), looking at abdomen deflection, found similar results wherein the magnitude of abdomen deflection increased with increasing sound intensity to 65 dB SPL but plateaued at stimuli 70 dB SPL and higher.

Responses from tethered locusts elicited with 80 dB SPL sounds presented from different speaker positions were statistically non-directional (Fig. 6D). Further, the locusts that were used for motor pattern and kinematic recordings (different animals than those used for experiments presented in Fig. 6) turned significantly more often to the left than to the right when stimulated with 88 dB SPL sounds from 90° to the left (binomial test,  $P=0.05$ ). We believe this was an unavoidable consequence of us necessarily using a rigid tether for our experiments. Dawson et al. (2004) have shown that free-flying locusts do produce negative phonotactic responses to 30-kHz pulse trains. These responses consist of turns away from the speaker but also include looping and spiralling flight, drops to the ground and rapid ascents; initial elements of which may include flight paths directed toward the source of the sound. Nonetheless, given the locusts were tethered and in the plane of our speaker, we expected our data to reflect the position of our speaker. That we did not see a statistically significant effect of speaker position could be due to many factors. For example, our high-speed camera required the use of bright lights during filming and locusts strongly orient to light in the environment and while flying (Uvarov 1977; Dawson et al. 2004); therefore, shadows and other visual asymmetries in the flight

chamber may have affected the direction of attempted turns. Also important is the fact we had to use a rigid tether that limits the movement of the locust during flight. A rigid tether removes the optical flow field, and interferes with mechano-sensory reafference, that would be present during free-flight. From several published studies employing rigid tethers, the presence of an auditory, thermal or visual (collision avoidance) stimulus on one side of a stationary flying locust does not exclusively produce turns away from the stimulus (Dawson et al. 1997; Robertson and Reye 1992; Shoemaker and Robertson 1998). This means that uncontrollable and unavoidable conditions present in, or caused by, the experimental arena may affect the choice of steering direction by locusts. Since these, and possibly other, unknown, factors are out of our control, we must evaluate the responses of the locusts to make sure they are steering responses to our stimulus and not artefact of the experimental setup. In this study, for each response we included in our analysis, we asked the following questions: (1) is the response to the stimulus and not something else, (2) is the response an attempt to turn, and (3) is the attempted turning response a coordinated response that would produce a change in flight path if the locust were not tethered?

The responses we observed were coordinated, stereotyped, reactions involving wing movements, leg and abdomen movement, and muscle activity and, as Table 2 shows that there was agreement between these different indices of steering direction. This means that although tethering affected the locusts "choice" of direction, we believe it did not affect the execution of the attempted steering response.

#### Motor pattern and wing kinematics

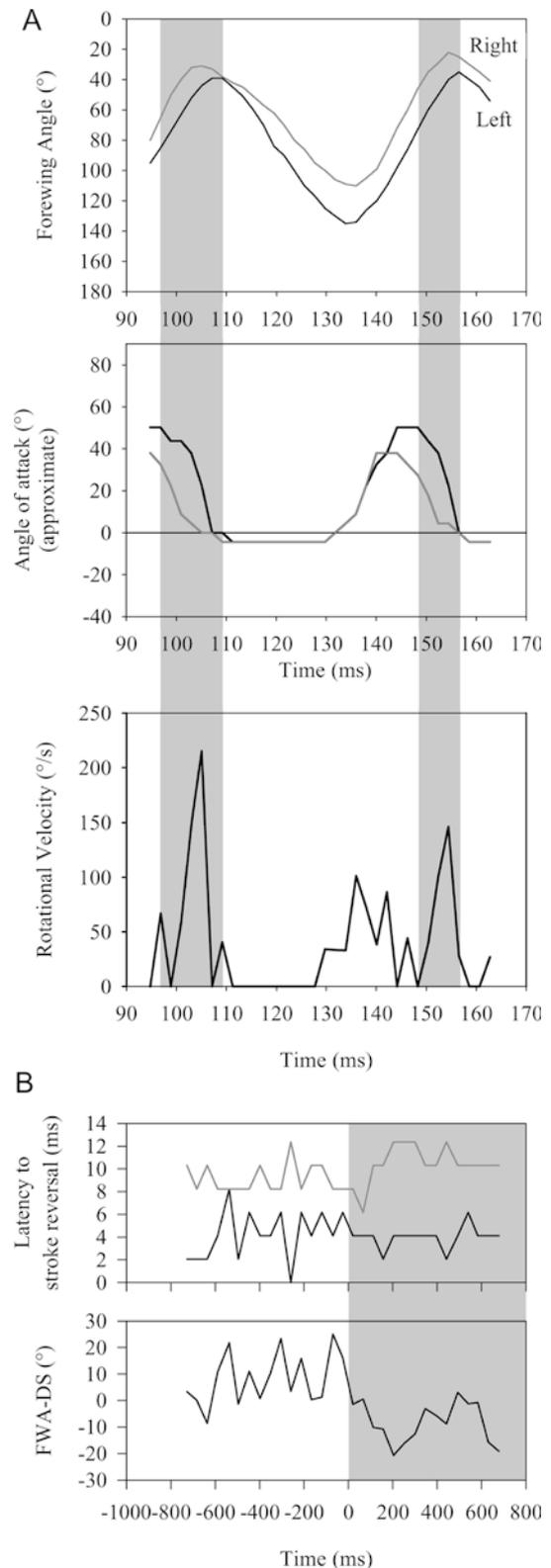
To understand how flight muscle activity produces changes in wing kinematics we returned to methods employed by numerous researchers nearly 30 years ago, namely, simultaneous multi-channel electromyography and wing position monitoring. During this intensive period of research, large advances were made in understanding steering responses by inducing changes in the three principal flight axes (yaw, roll, and pitch) and recording corresponding changes in muscle activity and wing kinematics (Zarnack 1988). These original studies isolated the principal flight axes by encouraging locusts to correctively steer to resume a straight course of flight using a variety of stimuli (both visual and mechano-sensory). A significant benefit of this approach is that locusts will sustain corrective postures for many (tens to hundreds) of cycles allowing motor and kinematic parameters to be averaged over a large number of cycles. Except perhaps for thermal avoidance steering (Shoemaker and Robertson 1998), intentional steering manoeuvres such as those involving auditory (and visual-collision course) stimuli are very abrupt, short duration, dynamic responses lasting typically less than a



**Fig. 12 A** Just before the beginning of the downstroke, the forewing flips (pronates) to establish the angle of attack for the downstroke (grey region illustrating period of left forewing flip). Throughout the downstroke the left wing (*black line*) is at a lower elevation than the right (*grey line*) showing this locust (L13R1) fictively turned left (segment starts 90 ms after stimulus onset). At the end of the upstroke, the right wing began to flip (pronate) before the left wing and both wings completed pronation before the downstroke began. The angle of attack was approximately  $-4.4^\circ$  during the downstroke for both left and right forewings and was between  $40^\circ$  and  $50^\circ$  during the upstroke before the flip began. The peak of the instantaneous velocity of rotation, which is between 100 and  $250^\circ \text{ s}^{-1}$ , occurs before the downstroke begins. **B** The wing on the outside of the turn flips earlier relative to its stroke reversal than the wing on the inside of the turn path. This data, from a different locust (L34R1) than above, also fictively turned to the left and shows that the right wing flip (*grey line*) is advanced in the cycle relative to the left. The changes in latency to the stroke reversal for the left and right forewings is coincident with the development of forewing asymmetry

dozen wing beat cycles. Further, a major difference between intentional and corrective steering is the extent of involvement of postural adjustments. During correctional steering of tethered locusts, subtle wing kinematic changes alone are often adequate to bring about sufficient changes in flight forces to restore “straight” flight; however, because leg and abdomen movements are routinely observed during intentional steering, it is not unreasonable to suspect motor patterns and wing movements to differ in magnitude from those seen during correctional steering. The motor pattern for intentional steering may also differ from the motor patterns recorded from locusts subjected to imposed rotations about the principle flight axes of yaw, roll and pitch because it is likely control of certain of these degrees of freedom are coupled (Taylor 2001).

We chose to examine responses on a cycle by cycle basis as opposed to correlating values derived by averaging a number of post- or pre-stimulus cycles. There is ample evidence that locusts can adjust their wing movements on a cycle by cycle basis (Wendler 1974; Burrows 1975; Reye and Pearson 1987, 1988) and for startle/escape responses it is not unreasonable to suspect that flight forces change rapidly. Our responses were abrupt and short in duration and these effects were apparent in both wing kinematics and motor patterns. This is consistent with observations of Möhl and Zar-



**Table 3** Summary of multiple pairwise correlations (Pearson product moment) between depressor muscle asymmetry and measured wing kinematic variables. Values in the cells are the number of significant ( $P < 0.05$ ) positive (+) correlations, significant negative (–) correlations, non-significant (N/C) correlations and the number of animals examined. *Asterisks* in the cells indicate significantly more positive than negative or significantly more negative than positive correlations than expected by chance (binomial test,  $P < 0.05$ ). The shading of the cells reflects situations (not statistical significance) where there are more positive than negative correlations (*light grey*) or more negative than positive correlations (*dark grey*); only cells with two or more significant correlations are coloured. The figure *inset* indicates important districts within the correlation matrix

nack (1977) who noted that responses to imposed roll could be generated within one or two cycles of stimulus onset.

Our model for the establishment of forewing asymmetry predicts significant correlations between forewing asymmetry measured during the downstroke, stroke

reversal asymmetry, and forewing asymmetry at the stroke reversal. Forewing asymmetry measured at one-third downstroke was positively correlated with forewing stroke reversal asymmetry (19 of 19 significant correlations were positive) and forewing asymmetry at the stroke reversal (16 of 16 significant correlations were positive). Further, we found strong correlations between these kinematics and depressor muscle timing asymmetries for all forewing muscles (M97, M98, and M99). Similar results were observed in the hindwing but we found fewer significant correlations. Hindwing asymmetry measured at one third downstroke was positively correlated with hindwing stroke reversal asymmetry (two of two significant correlations were positive) and hindwing asymmetry measured at the stroke reversal (two of three significant correlations were positive). Hindwing asymmetries were also positively correlated with M127 and M128 depressor asymmetries. These results support our model that bulk shifts in depressor timing is the motor pattern underlying asymmetric wing depression for both the forewings and hindwings.

Asymmetries in the hindwings were less pronounced than in the forewings and were opposite in direction (12 of 13 significant correlations were negative), meaning that during a left turn, the right hindwing was depressed at an angle lower than the left hindwing. This is reflected in correlations of hindwing first and second basalar activity with forewing motor or kinematic parameters. This occurrence is supported by observations of correctional steering to an artificial horizon (Thüring 1986). Hindwing subalar activity (M129) is advanced in the cycle on the inside of the turn path and bilateral asymmetries are positively correlated with forewing kinematics and forewing depressor muscle shifts. This suggests its role may not be to affect the timing of the stroke reversal but perhaps in establishing or maintaining hindwing angle of attack. The hindwing kinematics and negative correlations between M129 shifts and M127 shifts are supported by observations of Schmidt and Zarnack (1987).

Our motor pattern recorded during straight flight and during steering to an auditory stimulus is the same as the motor pattern recorded for thermal avoidance by Shoemaker and Robertson (1998). This is the most complete reconstruction of a motor pattern for intentional steering in locusts available for comparison. Recordings from M97, M98, M99, M127, and M85 during thermal avoidance showed that hindwing M127 activity leads forewing M97, M99, and M98 activity in that order (Shoemaker and Robertson 1998). This sequence of muscle contractions is the same order that we observed but we have additionally shown that hindwing M129 activity follows M127 but precedes M128 activity. As expected, hindwing muscle depolarizations precede forewing muscle depolarizations because the hindwing stroke leads the forewing stroke. After stimulation we found that all muscles retain their order of firing and advance in the cycle. This was also observed by Shoemaker and Robertson (1998) as well as that hindwing

M127 shifts opposite to M97 and recedes in the cycle. We found the same relationship in the shift of M127 pattern of shift but add that M127 recedes and can become coincident with M97 timing. Our observations, coupled with those of Shoemaker and Robertson (1998), support findings by Thüring (1986) that the hindwings can shift in opposite direction than the forewings during steering. Further, the order of activation of the hindwing muscles also stays the same so M129 activity follows M127 activity but precedes M128 activity.

The depressor shifts that we observed share many similarities with observations from Zarnack and Möhl (1977) for correctional steering. They found that locusts oscillated in yaw, pitch and roll in a wind stream shifted the times of spiking relative to the wing stroke cycle (calculated as the mean time of all active spikes for a cycle). When locusts were rotated in yaw, they found that M97, M98, M127, and M128 shifted together and fired earlier on the side ipsilateral to the turn and that M99 and M129 shifted together and fired later on the contralateral side. When locusts were pitched up, they found that muscles on left and right side shifted together and that M97 and M98 advanced in the cycle and M99 and M129 fired later. When locusts were rolled, they found that all forewing muscles on the side opposite the roll direction shifted to fire earlier and the muscles on the opposite side fired later. This was confirmed in a later study that calculated timing relative to M129 as a marker of the hindwing stroke (Waldmann and Zarnack 1988). In our experiments, we found that all forewing muscles on the inside of the turn fired earlier than their contralateral homologues which is similar to the above observations of locusts in yaw and roll but not pitch. These observations suggest that during intentional steering yaw and roll may not be independently modulated and may lead to tighter banked turns in free-flight.

During straight flight (i.e. the pre-stimulus period), when depressors were depolarized, they generally produced one or two spikes. After the stimulus, the number of spikes per burst increased in all depressors. Because the different depressors are innervated by different numbers of motor neurones (two neurones innervate M97, M99, M127, M128; one neurone innervates M98, M129) the increase in the number of spikes could be due to increases in recruitment or increases in the number of activations of each unit or a combination of both. Regardless, the number of spikes per burst is a measure of the strength of muscle contraction and may indicate the locust was attempting to increase the amount of thrust produced. Increases in thrust can also be produced by increases in wing beat frequency (Baker et al. 1981) which may be effected, wholly or in part, by an increase in spikes per burst.

The increase in spikes per burst occurred on both sides of the locust regardless of turn direction and this is in contrast to observations by Shoemaker and Robertson (1998) for thermal avoidance steering who described a "push-pull" relationship between contralateral depressors with the depressors on the inside of the turn

increasing in spikes per burst and the depressors on the outside of the turn path decreasing in spikes per burst. Other intentional steering studies have shown similar results for M97 to what this study has found (Baker 1979; Dugard 1967, Waldron 1967). Studies of correctional steering, however, have reported mixed results with respect to spikes per burst. Möhl and Zarnack (1977) observed an increased burst length with the muscle that fires earlier (on inside of turn). Others found that during roll, there was no change in burst length (Waldmann and Zarnack 1988; Schmidt and Zarnack 1987; Thüring 1986).

We believe that the functional consequences of the changes in wing movements effected by the motor pattern we describe above is to produce a combined yaw and roll torque in the direction of the more depressed forewing. The accompanying changes in abdomen deflection and abdomen dorsiflexion would augment the direction (and magnitude) of these torques. Abdomen deflection, by increasing drag on the side to which it is deflected (Camhi 1970) and by shifting the centre of mass of the locust (Zanker 1988), would contribute to the yaw and roll torques produced by the wings. Further, abdomen dorsiflexion, which would shift the centre of mass anterior may produce a downward pitching torque. The net result of all these contributions would be to produce a banked downward turn or spiral. Locusts in free flight have been observed making such manoeuvres in response to startling sounds (see Fig. 3 in Dawson et al. 2004).

#### Timing of the (dorsal) wing flip

We found evidence that locusts may actively control the timing of wing rotation and we believe this may be an additional mechanism for generating steering torques. As noted above, our measures are of the onset of the dorsal wing flip when the animal begins to change angle of attack at the end of the upstroke. Angles of attack during the downstroke were typical of those reported in the literature for locusts steering (Dugard 1967; Baker 1979; Zarnack 1988; Robertson and Reye 1992; Dawson et al. 1997) wherein we observed the left wing to be more pronated than the right wing during the downstroke. Although locusts initiated rotation of the right wing (at the end of the upstroke) before the left wing both before and after stimulation, the changes in the relative timing of the rotation with respect to the stroke reversal indicates that locusts may use this parameter for generating asymmetries in peak lift at the stroke transition.

In *Drosophila melanogaster*, a peak in lift is generated when the wingflip is completed before the beginning of wing translation and the magnitude of this lift peak is affected by changing the timing of the wing flip relative to the stroke reversal (Dickinson et al. 1999; Sane and Dickinson 2001). In our experiment, the forewing wing flip preceded downstroke translation (by approximately 8–15% of the cycle length). During straight flight, if the

forewings of locusts were generating lift by rotational circulation, then these forces would add to the forces generated during translation.

During steering, the forewing flip could contribute to the generation of steering torques in three ways. First, if the amount of lift generated by rotation of the left and right wings is the same, asymmetries in the elevation angles at the moment of the stroke reversal could affect the direction of the lift produced. Second, asymmetries in the magnitude of rotational lift may be generated by asymmetrically advancing the time of rotation relative to the stroke reversal. We showed that four of our five locusts (80%) that changed the timing of rotation of the left wing decreased the latency of rotation relative to the stroke reversal, and that seven of our twelve locusts (58%) that changed the timing of rotation of the right wing increased the latency of rotation relative to the stroke reversal. These observations are consistent with a strategy to either increase lift on the outside of the turn path or to decrease lift on the inside of the turn path. Third, asymmetries in the time that peak rotational lift is produced may affect the aerodynamic interaction between fore-hindwing pairs. All positive correlations between forewing pronation asymmetry (FW-PrA) and angular difference asymmetry (ADA) were significant (there were no negative correlations). There is likely to be considerable interaction between the forces generated by the forewings and hindwings owing to the pattern of airflow between the two wings during flight (Schmidt and Zarnack 1987; Schwenne and Zarnack 1987). Further, forewing pronation asymmetries were larger in magnitude than hindwing pronation asymmetries suggesting that the forewings may be the important control surfaces for establishing rapid direction changes.

#### Conclusions

In this paper, we have recorded from the direct downstroke flight muscles of the forewings and hindwings and measured differences in timing between contralaterally homologous muscles. As previously suggested (Dawson et al. 1997), asymmetric wing depression may contribute to the production of steering torques by affecting the left-right balance of forces generated by each wing; an imbalance that would add to any aerodynamic effects generated by angle of attack of the wings and changes in body posture. This study supports that hypothesis by showing that relative shifts in the timing of contralateral homologues are correlated with bilateral asymmetries in the timing of the stroke reversal, which in turn, are correlated with asymmetric wing depression. These results support previous predictions that forewing asymmetry results from bulk shifts in depressor timing that affect the timing of stroke reversals. This study also presents evidence that locusts may actively control the timing of the onset of wing rotation immediately before the stroke reversal. Future studies will have to confirm this observation and determine if the timing of wing

rotation contributes to generating steering torques. Finally, the time course of changes in wing asymmetry and depressor asymmetry in this study are the same as those reported in Dawson et al. (1997) and in previous studies of other intentional steering manoeuvres, namely collision avoidance (Robertson and Reye 1992; Robertson and Johnson 1993) and thermal avoidance (Robertson et al. 1996; Shoemaker and Robertson 1998) suggesting that locusts employ a common motor program for effecting rapid avoidance/escape movements.

**Acknowledgements** We are indebted to Prof. Dr. Wolfram Kutsch, and especially Holger Martz, for showing us an improved method for electromyography of locust flight muscles. Ron Harding built our 16-channel differential amplifier and wrote software for digitizing wing kinematics from AVI files of our high-speed digital cinematography data; contributions without which this study would not have been possible. We thank Dr. James Fullard for loaning us acoustic equipment for calibrating our speakers and sound source. We also thank the anonymous reviewers of this manuscript for their constructive criticism. This study was supported by grants from the Natural Sciences and Engineering Research Council of Canada (NSERC) to R.M.R. The experiments described in this manuscript comply with the *Principles of animal care*, publication No. 86-23, revised 1985, of the National Institutes of Health and also with the current laws of Canada.

## References

- Baker PS (1979) The role of forewing muscles in the control of direction in flying locusts. *J Comp Physiol A* 131:59–66
- Baker PS, Gewecke M, Cooter RJ (1981) The natural flight of the migratory locust *Locusta migratoria* L. III. Wing-beat frequency, flight speed and attitude. *J Comp Physiol A* 141:233–237
- Burrows M (1975) Monosynaptic connexions between wing stretch receptors and flight motoneurons of the locust. *J Exp Biol* 62:189–219
- Burrows M (1996) The neurobiology of an insect brain. Oxford University Press, Oxford
- Camhi JM (1970) Yaw-correcting postural changes in locusts. *J Exp Biol* 52:519–531
- Dawson JW, Dawson-Scully K, Robert D, Robertson RM (1997) Forewing asymmetries during auditory avoidance in flying locusts. *J Exp Biol* 200:2323–2335
- Dawson JW, Kutsch W, Robertson RM (2004) Auditory evoked evasive manoeuvres in free-flying locusts and moths. *J Comp Physiol A* 190:69–84
- Dickinson MH, Lehmann F, Sane SP (1999) Wing rotation and the aerodynamic basis of insect flight. *Science* 284:1954–1960
- Dudley R (2002) Mechanisms and implications of animal flight maneuverability. *Integr Comp Biol* 42:135–140
- Dugard JJ (1967) Directional change in flying locusts. *J Insect Physiol* 13:1055–1063
- Hoy R, Nolen T, Brodfuehrer P (1989) The neuroethology of acoustic startle and escape in flying insects. *J Exp Biol* 146:287–306
- Möhl B, Zarnack W (1977) Activity of the direct downstroke flight muscles of *Locusta migratoria* (L.) during steering behaviour in flight. II. Dynamics of the time shift and changes in the burst length. *J Comp Physiol A* 118:235–247
- Nachtigall W (1989) Mechanics and aerodynamics of flight. In: Goldsworthy GJ, Wheeler CH (eds) *Insect flight*. CRC Press, Boca Raton
- Reye DN, Pearson KG (1987) Projections of the wing stretch receptors to central flight neurons in the locust. *J Neurosci* 7:2476–2487
- Reye DN, Pearson KG (1988) Entrainment of the locust central flight oscillator by wing stretch receptor stimulation. *J Comp Physiol A* 162:77–89
- Robert D (1989) The auditory behaviour of flying locusts. *J Exp Biol* 147:279–301
- Robert D, Rowell CHF (1992) Locust flight steering. II. Acoustic avoidance manoeuvres and associated head movements, compared with correctional steering. *J Comp Physiol A* 171:53–62
- Robertson RM, Johnson AG (1993) Collision avoidance of flying locusts: steering torques and behaviour. *J Exp Biol* 183:35–60
- Robertson RM, Reye DN (1992) Wing movements associated with collision-avoidance manoeuvres during flight in the locust *Locusta migratoria*. *J Exp Biol* 163:231–258
- Robertson RM, Kuhnert CT, Dawson JW (1996) Thermal avoidance during flight in the locust *Locusta migratoria*. *J Exp Biol* 199:1383–1393
- Rowell CHF (1988) Mechanisms of flight steering in locusts. *Experientia* 44:389–395
- Sane SP, Dickinson MH (2001) The control of flight force by a flapping wing: lift and drag production. *J Exp Biol* 204:2607–2626
- Schmidt J, Zarnack W (1987) The motor pattern of locusts during visually induced rolling in long-term flight. *Biol Cybern* 56:397–410
- Schwenne T, Zarnack W (1987) Movements of the hindwings of *Locusta migratoria*, measured with miniature coils. *J Comp Physiol A* 160:657–666
- Shoemaker KL, Robertson RM (1998) Flight motor patterns of locusts responding to thermal stimuli. *J Comp Physiol A* 183:477–488
- Snodgrass RE (1929) The thoracic mechanism of a grasshopper and its antecedents. *Smithson Misc Coll* 82:1–111
- Sokal RL, Rohlf FJ (1981) *Biometry: the principles and practice of statistics in biological research*, 2nd edn. Freeman, New York
- Stapells DR, Picton TW, Smith AD (1982) Normal hearing thresholds for clicks. *J Acoust Soc Am* 72:74–79
- Taylor GK (2001) Mechanics and aerodynamics of insect flight control. *Biol Rev* 76:449–471
- Thüring DA (1986) Variability of motor output during flight steering in locusts. *J Comp Physiol A* 158:653–664
- Uvarov BP (1977) *Grasshoppers and locusts: a handbook of general acridology*, vol 2. Behaviour, ecology, biogeography, population dynamics. Cambridge University Press, Cambridge and Centre for Overseas Pest Research, London
- Waldmann B, Zarnack W (1988) Forewing movements and motor activity during roll manoeuvres in flying desert locusts. *Biol Cybern* 59:325–335
- Waldron I (1967) Neural mechanism by which controlled inputs influence motor output in the flying locust. *J Exp Biol* 47:213–228
- Wendler G (1974) The influence of proprioceptive feedback on locust flight coordination. *J Comp Physiol* 88:173–200
- Wilson DM (1968) The nervous control of flight and related behaviour. *Adv Insect Physiol* 5:289–338
- Zanker JM (1988) How does lateral abdomen deflection contribute to flight control of *Drosophila melanogaster*. *J Comp Physiol A* 162:581–588
- Zar JH (1984) *Biostatistical analysis*, 2nd edn. Prentice-Hall, Englewood Cliffs, New Jersey
- Zarnack W (1988) The effect of forewing depressor activity on wing movement during locust flight. *Biol Cybern* 59:55–70
- Zarnack W, Möhl B (1977) Activity of the direct downstroke flight muscles of *Locusta migratoria* (L.) during steering behavior in flight. I. Patterns of time shift. *J Comp Physiol A* 118:215–233