Flexibility and control of thorax deformation during hawkmoth flight

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The interaction between neuromuscular systems and body mechanics plays an important role in the production of coordinated movements in animals. Lepidopteran insects move their wings by distortion of the thorax structure via the indirect flight muscles (IFMs), which are activated by neural signals at every stroke. However, how the action of these muscles affects thorax deformation and wing kinematics is poorly understood. We measured the deformation of the dorsal thorax (mesonotum) of tethered flying hawkmoths, *Agrius convolvuli*, using a high-speed laser profilometer combined with simultaneous recordings of electromyograms and wing kinematics. We observed that locally amplified mesonotum deformation near the wing hinges ensures sufficient wing movement. Furthermore, phase asymmetry in IFM activity leads to phase asymmetry in mesonotum oscillations and wingbeats. Our results revealed the flexibility and controllability of the single structure of the mesonotum by neurogenic action of the IFMs.

1. Introduction

How the nervous system, muscles and body mechanics interact to generate coordinated movement is a central theme in understanding the mechanisms of animal behaviour. Flying insects are useful models, because they have evolved sophisticated neuromuscular and mechanical systems for wing motion [1–3]. Furthermore, the thoracic designs powering dorsal–ventral wing movements are diverse among insect orders [1]. Lepidoptera have characteristic mechanical and control designs. Their dorsal–ventral wing movement is generated by the action of indirect flight muscles (IFMs) that primarily deform the dorsal thorax (mesonotum), a mechanism that is advantageous for aiding the elastic and resonance properties of the thorax (figure 1a) [1,2]. The IFMs act as the dominant power generators in Coleoptera, Hymenoptera and Diptera as well as in Lepidoptera. In terms of muscular control, the lepidopteran IFMs are activated by motoneurons at every stroke (synchronous or neurogenic muscle), whereas IFMs in Coleoptera, Hymenoptera and Diptera are activated by mechanical stretch and are not synchronized with motoneuron activities (asynchronous muscle) [1,2]. Therefore, Lepidoptera can use the neurogenic controllability of the IFMs for finer control of wing kinematics, while at the same time gaining mechanical benefits. Previous studies of hawkmoths have shown that phase asymmetry in the activity of bilateral IFMs can generate asymmetry in the power and kinematics of the wing beat [4–6]. To achieve such asymmetrical control, the single structure of the mesonotum can be locally deformed and transmit asymmetrical power to the wings. However, it is poorly understood how neurogenic activities in the IFMs produce different local deformations of the mesonotum and modulate wing kinematics. In this study, we elucidated the local deformation of the mesonotum in the hawkmoth (*Agrius convolvuli*) via imaging with a high-speed two-dimensional profilometer, further combined with simultaneous measurements of electromyograms and wing kinematics.
2. Material and methods

(a) Experimental conditions
Adult male hawkmoths (*A. convolvuli*; morphological parameters are shown in the electronic supplementary material, table S1) were ventrally tethered to the tip of a rigid post after removal of scales on the thorax and all legs (figure 1b). The longitudinal body axis was horizontally aligned along the y-axis. A high-speed laser profilometer (LI-Y7060, Keyence, Osaka, Japan; wavelength 405 nm), placed above the moth, acquired two-dimensional profiles of the mesonotum at 2000 profiles per second. A high-speed camera (Fastcam 512PCI, Photron, Tokyo, Japan) with infrared illumination captured the wing elevation of the moth in posterior view at 2000 frames per second. To elicit fictive flight manoeuvres (optomotor response), we presented optic flow stimuli (moving red and white sinusoidal gratings) on two LCD monitors. All experiments were conducted at 25–30°C under dark conditions (illuminance: 75 lux).

(b) Experiment 1: three-dimensional measurement of thorax deformation
Three-dimensional thorax deformation was measured with the profilometer by scanning over the mesonotum (scutum and scutellum, divided by a suture) along the body axis (y-axis), during upward or downward fictive flight elicited by the optic flow (horizontal gratings, contrast frequency: 7.59 Hz, spatial frequency: 0.067 cycles deg$^{-1}$). Forewing tegulae, the flat structures covering the lateral edge of the mesonotum (figure 1c), were bilaterally removed to expose the mesonotum in experiment 1 ($n = 8$); removal of tegulae did not alter wing kinematics; see the electronic supplementary material, figure S1, table S2 and movie...
The wing stroke phase, beginning with the wing in the uppermost position, was determined from the wing elevation angle (the angle between the leading edge of the forewing projected onto the x–z-plane and the horizontal x–y-plane). The y-position of the mesonotum in each mesh cell (160 (x) × 250 (y) μm) was reconstructed every 10° in the stroke phase. The z-displacement of the mesonotum relative to the resting position was calculated by subtracting the z-position at rest from the position during wing flapping. For quantification, we measured the amplitude and mean angle of wing elevation, and the amplitude and mean z-displacement of mesonotum oscillation at five points (sc1: centre of the scutum; sc2–4: posterior of the scutum, x–y-plane). st1: centre of the scutellum (figure 1). The mean angle of wing elevation, and the amplitude and mean z-displacement of mesonotum oscillation at five points (sc1: centre of the scutum; sc2–4: posterior of the scutum, x–y-plane). sc2–4: posterior of the scutum; sc4 was averaged; st1: centre of the scutellum (figure 1).

(c) Experiment 2: transmission of phase asymmetry from IFMs to the wing

Electromyograms were recorded from the bilateral indirect depressor (dorsal longitudinal muscle: DLM, figure 1a) and elevator muscles (dorsal ventral muscle: DVM). Scutum displacement on each side was determined at a position with distance d from the midline (d = 0.2–1.4 mm; figure 2a). We left the forewing tegulae intact in experiment 2 as they did not obscure the measured positions (n = 6). To induce phase asymmetry in the bilateral IFMs, we presented laterally oscillating optic flow (vertical gratings oscillated at 1 Hz, spatial frequency: 0.05 cycle deg−1, amplitude: 25°) to elicit fictive turning flight [4]. We detected the timings of representative physiological and kinematic events, including the peak amplitude of the first spike in DLM and DVM activities, the lowermost (S1) and uppermost (S2) scutum oscillation, and the corresponding wing elevation at the uppermost (W1) and lowermost positions (W2) of each cycle. The time differences between bilateral events were normalized by the corresponding cycle length of the DLM (qDLM; mean length between bilateral DLMs) and represented as a phase difference (Δθ, figure 2b). We fitted a trigonometric function to the time course of Δθ using a linear mixed-effects model (LME) and calculated the amplitude of the fitted model (Δθ amplitude) as an index of phase asymmetry.

For detailed procedures, see the electronic supplementary material.

Figure 2. Transmission of phase asymmetry from IFMs to the wing. (a) Measurement points for bilateral z-displacement on the scutum (arrowheads), d: distance from the midline. (b) Sample recording and definition of temporal parameters. Vertical, dashed lines indicate timings of events on the left (L) and right (R). ηDLM: mean cycle length between bilateral DLMs (shaded area); Δθ: phase difference between bilateral events (red—downstroke-onset events: ΔθL; DLM; ΔθR, scutum lowermost; ΔθhL, wing uppermost; blue—upstroke-onset events: ΔθhL, DVM; ΔθhR, scutum uppermost; ΔθhR, wing lowermost). Positive Δθ indicates a phase delay of a left event. (b) Sample recording of the phase difference in the downstroke-onset events (left) and upstroke-onset events (right) during laterally oscillating optic flow (stim.). Open circles: ΔθL and ΔθhL; closed circles: ΔθR and ΔθhR (d = 1 mm); plus signs: ΔθL and ΔθhL, ΔθR and ΔθhR (d). (c) Δθ amplitude of all events. Scatter plots with regression lines indicate ΔθL and ΔθhL at different points (d). Solid horizontal lines: ΔθhL and ΔθhR; dashed horizontal lines: ΔθL and ΔθR. All Δθ amplitudes were significant (p < 0.001; 358–363 strokes, n = 6). (Online version in colour.)
(paired t-test, \( p < 0.001 \)). Corresponding significant dorsal and ventral shifts of the mean \( z \)-displacement were observed in the scutum (sc1–4: \( p < 0.01 \); figure 1f; and electronic supplementary material, figure S2), whereas they were not observed in the scutellum (st1, \( p = 0.71 \)). These results indicate that scutum \( z \)-displacement is more involved in the dorsal–ventral shift of the wings than is the scutellum.

We subsequently investigated the transmission of phase asymmetry from the bilateral IFMs (spike timing difference, within approx. 6 ms) to the wings via scutum deformation (experiment 2). In response to laterally oscillating optic flow, hawkmoths performed fictive turning manoeuvres (electronic supplementary material, movie S3) and \( \Delta \theta \) in all events varied sinusoidally (figure 2c). The \( \Delta \theta \) amplitudes were significant in all physiological and kinematic events (LME, \( p < 0.001 \); electronic supplementary material, table S3), although \( \Delta \theta \) amplitudes of the scutum and the wings were reduced compared with those of IFMs (e.g. \( \Delta \theta_{\text{st1}} \): 19.2°, \( \Delta \theta_{\text{sh}} \): 2.1–5.2° (\( d = 0.2–1.4 \text{ mm} \)), and \( \Delta \theta_{\text{w1}} \): 6.4°; figure 2d). Furthermore, the \( \Delta \theta \) amplitudes of the upstroke-onset events were larger than those of downstroke-onset events, which also appeared as larger \( \Delta \theta_{\text{st}} \) amplitudes at distal positions (larger \( d \), i.e. near the DVM; figure 2e) compared with \( \Delta \theta_{\text{sh}} \) amplitudes. These results indicate that the bilateral IFMs, particularly the DVMs, have the capability to modulate phase asymmetry in wing strokes via asymmetrical deformation of the scutum.

In this study, we demonstrated the spatio-temporal mechanical properties of the single structure of the mesonotum. The possible factors involved in localized deformation are the morphological properties of the mesonotum, such as the details of its hemispherical shape, the sutures on the surface and the arrangements of the IFMs [1–3]. The inward–outward movement of the scutum is consistent with earlier observations in Diptera and Hymenoptera [2,7,8]. These insects use asynchronous IFMs for power generation, whereas small synchronous steering muscles effectively alter the dynamics of the wing hinge at every stroke [9]. Conversely, in Lepidoptera, our results suggest that localized deformation of the scutum under the neurogenic control of the synchronous IFMs alters the wing hinge structure, which is then further altered by the direct action of steering muscles.

The phase control of muscle activation within a contraction cycle is important for power modulation [4]. Therefore, the transmission of phase asymmetry is not a straightforward process even at the muscular level and would be further reduced owing to the mechanical coupling of the bilateral scutum. Nevertheless, the capability of IFMs to control the wings asymmetrically indicates that the scutum can accept the local inputs of IFM contractions and transmit them to the wing. In particular, the DVMs, located near the wing hinges, would effectively deform the lateral scutum and control the wings. Recent studies have reported the contribution of the power-producing IFMs to lateral control in both the synchronous [4–6] and the asynchronous types [10]. The multiple functions of the IFMs provide flexibility for flight control and will be further elucidated by a combination of morphometry, physiology and computational structural analysis.

**Ethics.** Animal experiments were performed according to MEXT guidelines.

**Data accessibility.** Supplemental methods, figures, tables and movies are included in the electronic supplementary material. Datasets of thorax morphology are available on Dryad: http://dx.doi.org/10.5061/dryad.310nt.

**Authors’ contributions.** N.A. performed the experiments. Both the authors designed the experiments, wrote the manuscript, approved the final version of the manuscript and are accountable for its contents.

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**References**


