



## Research

**Cite this article:** Hall JM, McLoughlin DP, Kathman ND, Yarger AM, Mureli S, Fox JL. 2015 Kinematic diversity suggests expanded roles for fly halteres. *Biol. Lett.* **11**: 20150845. <http://dx.doi.org/10.1098/rsbl.2015.0845>

Received: 7 October 2015

Accepted: 3 November 2015

### Subject Areas:

behaviour, biomechanics, neuroscience

### Keywords:

halteres, kinematics, flight

### Author for correspondence:

Jessica L. Fox

e-mail: [jlf88@case.edu](mailto:jlf88@case.edu)

<sup>†</sup>These authors contributed equally to this study.

Electronic supplementary material is available at <http://dx.doi.org/10.1098/rsbl.2015.0845> or via <http://rsbl.royalsocietypublishing.org>.

# Kinematic diversity suggests expanded roles for fly halteres

Joshua M. Hall<sup>†</sup>, Dane P. McLoughlin<sup>†</sup>, Nicholas D. Kathman, Alexandra M. Yarger, Shwetha Mureli and Jessica L. Fox

Department of Biology, Case Western Reserve University, Cleveland, OH 44106-7080, USA

The halteres of flies are mechanosensory organs that provide information about body rotations during flight. We measured haltere movements in a range of fly taxa during free walking and tethered flight. We find a diversity of wing–haltere phase relationships in flight, with higher variability in more ancient families and less in more derived families. Diverse haltere movements were observed during free walking and were correlated with phylogeny. We predicted that haltere removal might decrease behavioural performance in those flies that move them during walking and provide evidence that this is the case. Our comparative approach reveals previously unknown diversity in haltere movements and opens the possibility of multiple functional roles for halteres in different fly behaviours.

## 1. Introduction

Fly flight is enabled in part by halteres, mechanosensory organs that detect body rotations [1,2]. Halteres are homologous to hindwings [3] and are oscillated at wingbeat frequency during flight. Haltere neurons are sensitive to small movements and not specialized for particular frequencies [4]; thus, any movement of the haltere may be detected by the nervous system. Characterizing these movements is essential to understanding the information that halteres provide to the fly's nervous system.

In four-winged insects, hindwings are often coupled to front wings [5], but in the small number of flies that have been observed, the halteres oscillate out of phase with the wings [6]. The wings possess the same type of mechanosensory afferents as the halteres [7,8], and the relative timing of wing and haltere nerve activity may be essential to steering [9,10]. Many insects generate flight manoeuvres by altering the phase of front and hindwing oscillations. It is possible that some fly groups retain this capability, with important consequences for flight control, but this has not been measured.

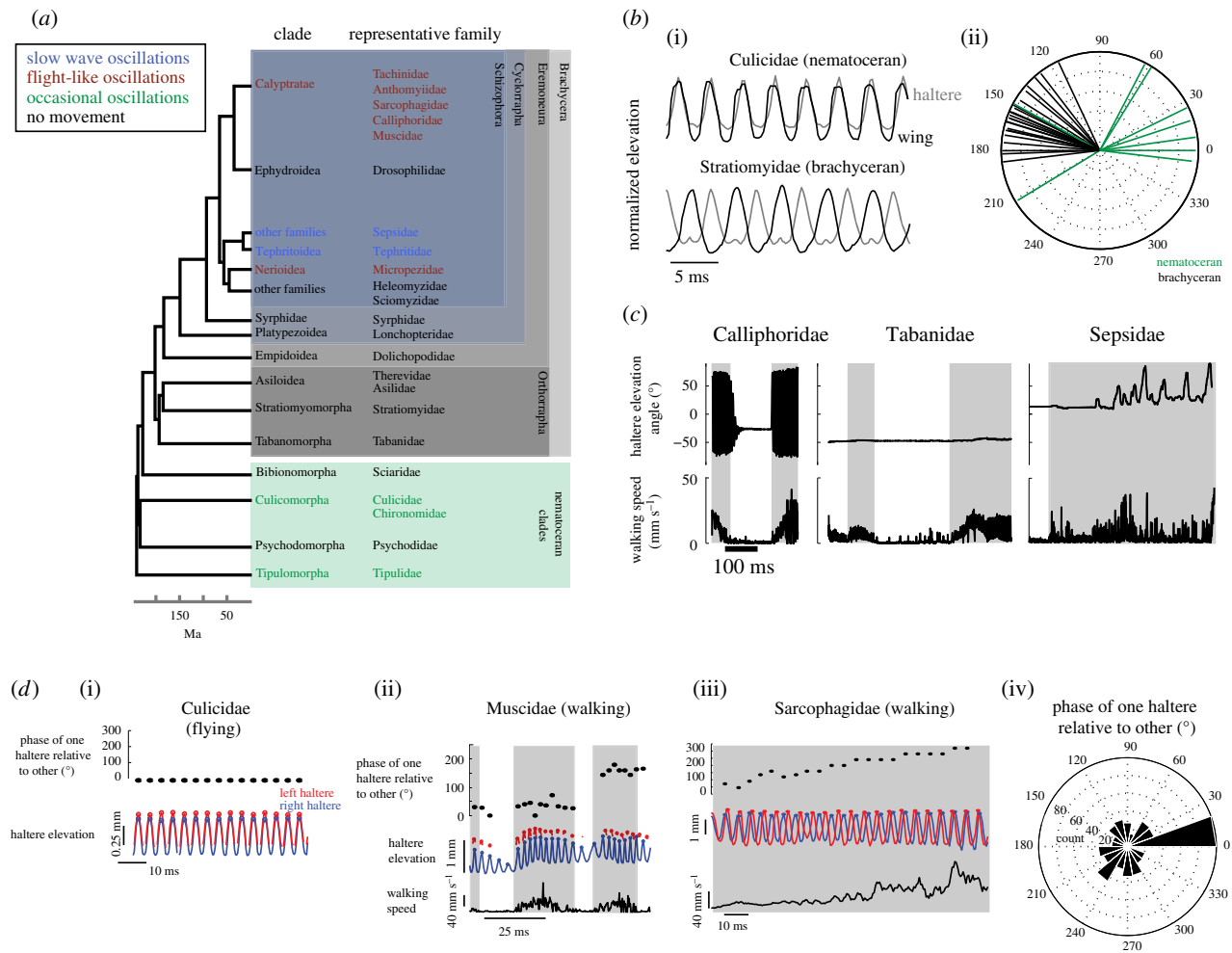
Still less is known about haltere function when flies are on the ground. There are reports of haltere movements during walking in some flies [11–13], but these observations were not detailed, and were limited to only two families. What are the kinematics of these movements, and are they relevant for fly behaviour?

Here, we observed haltere movements during flying and walking behaviour across several fly families (figure 1*a*). Our observations show a large diversity of haltere movements in flight and in walking, suggesting that halteres have a role in fly behaviour beyond that known in flight.

## 2. Results and discussion

### (a) During flight, the phase of the haltere stroke relative to the wing stroke varies across species

We filmed 41 flies (26 families) in tethered flight (table 1). All flies in the brachyceran (short-antennaed) suborder oscillated their halteres near 180° with respect



**Figure 1.** Diversity of haltere movement during flight and walking. (a) Phylogeny of Diptera after Wiegmann *et al.* [14]. Families are colour-coded according to haltere movements during walking. (b) (i) Traces of haltere and wing elevations in flight for a nematoceran (top) and brachyceran fly (bottom). (ii) Haltere oscillation phases relative to wingbeat for flies shown in table 1. The phase is represented by the angle of each line and the length of each line represents vector strength. (c) Raw data traces from videos of walking flies. Top trace: haltere elevation angle. Bottom trace: horizontal body velocity. (d) Phase relationships between the two halteres are different during walking and flying. (i) The two halteres of flying flies are consistently in phase ( $0^\circ$ ) with each other. Top: Phases of the left haltere (bottom, red) relative to the right (bottom, blue). Circles denote elevation peaks. (ii) The oscillation phases can change rapidly during walking. A housefly (*Muscidae*) shows a switch between in-phase haltere oscillations and out-of-phase haltere oscillations. Data were captured with a single camera and thus, both haltere bases were not visible in all frames. Grey boxes designate walking bouts. (iii) A two-camera video capturing both halteres shows a gradual shift in phase. (iv) Rose plot of the distribution of relative phases of one haltere to the other includes the entire oscillation cycle,  $n = 7$  trials in four flies.

to the wings, but nematoceran (long-antennaed) flies showed a variety of phase relationships (figure 1b). Mosquitoes and midges oscillated their halteres nearly in phase with the wings (table 1; figure 1b). The sensilla on both halteres and wings fire phase-locked spikes in each oscillation [4,7]. If the phases of haltere movement vary, relative phases of spiking in wing and haltere sensilla will also vary, requiring different decoding strategies in the central nervous system. Nematoceran families are ancient relative to brachyceran families, suggesting that mechanisms for wing–haltere coordination evolved after the halteres themselves.

**(b) Haltere movements during walking are correlated with phylogeny**

Patterns of haltere movement in walking flies were dependent on phylogeny (figure 1a; [14]). In most families, halteres do not move during standing or walking. These families include flies that diverged from their ancestor over 200 Ma (*Sciaridae*) to more recent families diverging less than 100 Ma (*Drosophilidae*). The absence of haltere movements suggests that for

diverse flies, the haltere nerve is silent during walking. In sharp contrast, flies in the calyptate families (*Muscidae*, *Anthomyiidae*, *Calliphoridae*, *Sarcophagidae* and *Tachinidae*) and one acalyptate fly (*Micropedizae*) always oscillated their halteres during walking (figure 1c). These walking oscillations were similar in frequency and amplitude to the oscillations in tethered flight in the same individuals.

Flies in the families *Tephritidae* and *Sepsidae* moved their halteres while walking and standing. These movements are much slower than wingbeat frequency, and not sinusoidal (figure 1c). *Tephritid* flies have patterned wings that they wave slowly in communication to conspecifics and predators [15]. We find that the halteres move in similarly non-rhythmic ways, probably providing unique patterns of sensory information.

**(c) Flies change the phase relationship between the two halteres during walking**

In flight, halteres always oscillate in phase with each other, even at high wingbeat frequencies (figure 1d(i)). However, in walking, calyptate flies can change the phase of the two

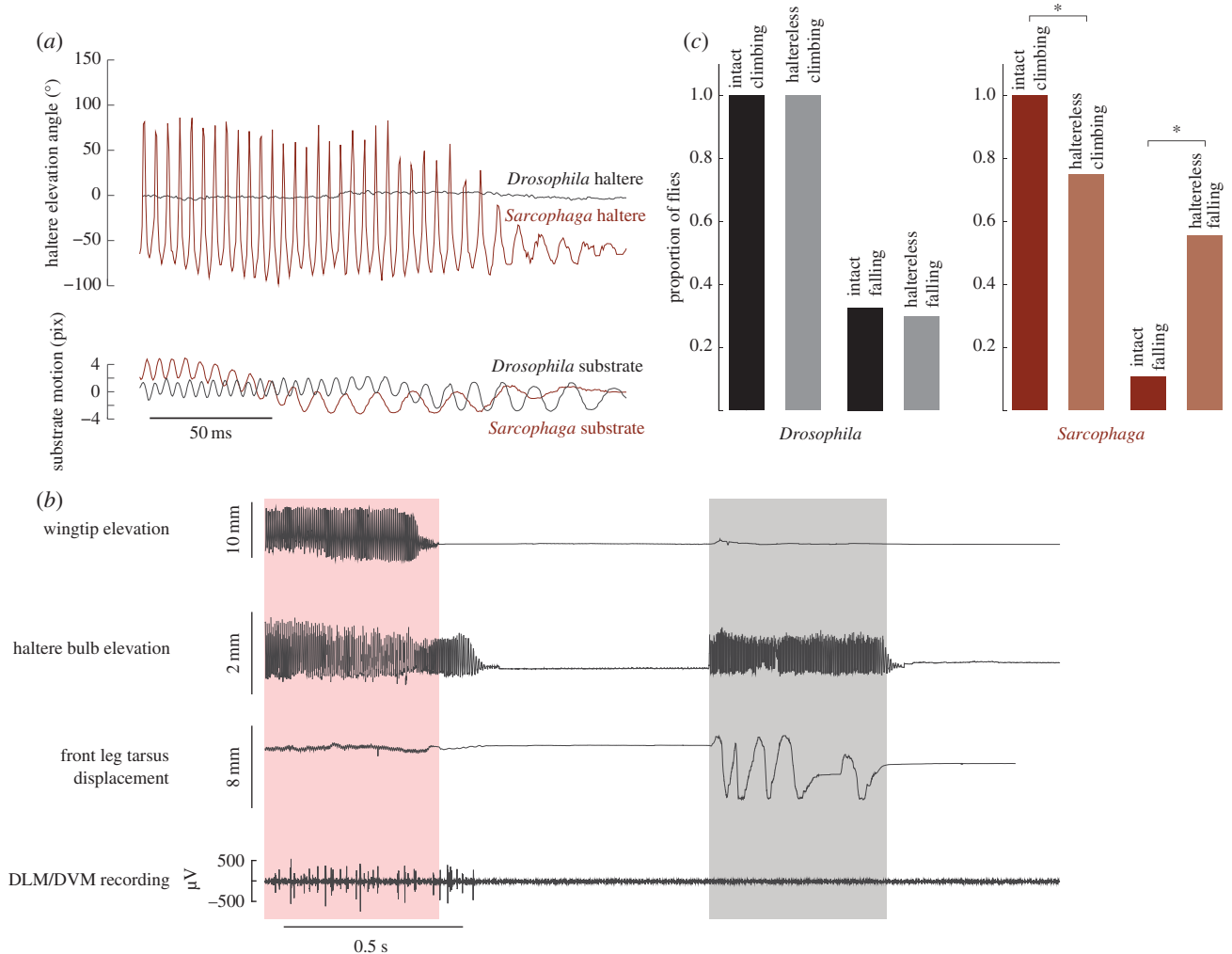
**Table 1.** Measurements of various parameters of flight motion for 41 flies in 26 families. Each row represents a unique individual fly performing a single bout of flight.

suborder	family	phase of haltere relative to wing (deg)	vector strength	wingbeat frequency (Hz)	no. wingbeats analysed
Brachycera	Asilidae	157	0.99	137 ± 0.8	22
Brachycera	Calliphoridae	141	0.99	185.6 ± 1.1	21
Brachycera	Calliphoridae	146	1	160.6 ± 1.6	21
Brachycera	Calliphoridae	159	0.98	199.3 ± 1.5	21
Brachycera	Calliphoridae	130	0.98	200.8 ± 1.8	21
Brachycera	Chamaemyiidae	160	0.96	262.9 ± 2.6	24
Brachycera	Chloropidae	175	0.98	202.8 ± 2.1	22
Brachycera	Dolichopodidae	127	0.98	167.8 ± 1.2	22
Brachycera	Dolichopodidae	148	0.95	152.3 ± 1.4	23
Brachycera	Dolichopodidae	154	0.97	244.2 ± 3	21
Brachycera	Dolichopodidae	115	0.99	166.8 ± 1.9	22
Brachycera	Drosophilidae	152	0.99	207.7 ± 0.9	26
Brachycera	Heleomyzidae	180	0.99	92.5 ± 0.8	21
Brachycera	Heleomyzidae	159	0.98	185.8 ± 1.2	22
Brachycera	Lauxaniidae	168	0.96	170.5 ± 1.8	23
Brachycera	Lonchopteridae	163	0.99	102.3 ± 0.9	21
Brachycera	Muscidae	132	0.99	177 ± 1.6	22
Brachycera	Phoridae	160	0.98	90.1 ± 0.9	21
Brachycera	Pipunculidae	171	0.98	269.6 ± 2.3	21
Brachycera	Sarcophagidae	168	0.98	168.9 ± 2.1	21
Brachycera	Sciomyzidae	167	0.96	151.5 ± 2.4	21
Brachycera	Sepsidae	174	0.99	215.1 ± 2.1	22
Brachycera	Stratiomyidae	182	0.99	109.7 ± 0.9	22
Brachycera	Stratiomyidae	157	0.99	131.6 ± 1	21
Brachycera	Stratiomyidae	157	0.99	136.7 ± 0.8	21
Brachycera	Syrphidae	164	0.98	185.3 ± 1.6	24
Brachycera	Syrphidae	141	0.96	202.8 ± 3	22
Brachycera	Tabanidae	187	0.99	148.9 ± 1.2	21
Brachycera	Tabanidae	129	0.99	102.7 ± 0.9	21
Brachycera	Tachinidae	156	0.97	179.5 ± 2.3	22
Brachycera	Tephritidae	163	0.99	157.9 ± 1.5	21
Brachycera	Therevidae	138	0.99	106.3 ± 0.7	17
Nematocera	Chironomidae	351	0.94	218.4 ± 1.8	47
Nematocera	Chironomidae	0	0.97	445.4 ± 4.5	51
Nematocera	Culicidae	24	0.98	286.1 ± 2.3	22
Nematocera	Culicidae	18	0.97	298.4 ± 3.9	27
Nematocera	Culicidae	26	0.98	339.1 ± 2.7	21
Nematocera	Psychodidae	152	0.99	114.3 ± 0.8	21
Nematocera	Sciaridae	211	0.97	161.7 ± 1.1	21
Nematocera	Tipulidae	58	0.99	57 ± 0.4	20
Nematocera	Tipulidae	62	0.98	48.3 ± 0.5	18

halteres rapidly from stroke to stroke (figure 1*d*(ii,iii)). We show the distribution of relative haltere phases for 545 oscillations in calyptate flies (figure 1*d*(iv)). The relative phases between the two halteres span the entire cycle, showing

that they can take on all possible phase relationships and are not limited to in-phase or out-of-phase oscillations.

This behaviour is very different from the in-phase oscillations observed in all flying flies (figure 1*d*(i)). Because



**Figure 2.** Haltere movements while walking and standing on unstable surfaces are similar, and they do not require direct flight muscle activity. (a) Haltere movements during perturbations. Top: *Sarcophaga* shows large haltere oscillations during perturbations; *Drosophila* does not. Bottom: movement of the substrate. (b) Behaviour and dorsal longitudinal muscle/dorsal–ventral muscle (DLM/DVM) activity in a *Sarcophaga* fly during flying (red-shaded box, first) and walking (grey-shaded box, second). Top trace: the wing is oscillated during flight and stationary during walking. Second trace: the haltere moves during both walking and flying, with similar amplitude in each. Third trace: the front leg makes large movements only during walking. Bottom trace: DLM/DVM are only active during flight. (c) Halteres influence the proportion of flies climbing a vertical wall and falling from the wall after a perturbation, but only in species that oscillate their halteres while walking. Left: *Drosophila* does not oscillate its halteres while walking, and haltere ablation has no effect on the proportion of flies climbing or falling. Right: *Sarcophaga* oscillates its halteres during walking. Haltere ablation decreases the proportion of flies climbing and increases the proportion falling. (Online version in colour.)

haltere afferent neurons are exquisitely sensitive to phase [16], the change in phase indicates that spike arrival times will be more variable during walking than during flight.

#### (d) Haltere movements during perturbations are similar to haltere movements during walking

To determine if flies actively oscillate their halteres when the substrate becomes unstable, we gently vibrated the surface on which they stood. We tested one species that does not move its halteres while walking (*Drosophila melanogaster*) and one species that does (*Sarcophaga bullata*). When challenged with a vibrating substrate, *Sarcophaga* oscillated its halteres, at approximately the same frequency as in flight, in eight of 12 trials. *Drosophila* never moved its halteres (5 of 5 trials; figure 2a).

#### (e) Haltere movements do not require activation of indirect flight muscles

Are haltere movements in wing-clipped, walking flies the result of a frustrated take-off attempt? We show that they are

not. First, we observed no thoracic movement during haltere movements in walking. Second, recordings of indirect flight muscles (dorsal longitudinal muscle and dorsal–ventral muscle) showed that they are active during wing movement only, and that haltere movements during walking occur without this activation (figure 2b). The halteres of walking flies are thus not moved by thoracic movements, as in flight [6,9], but rather by muscles of the halteres themselves [9,17].

#### (f) Haltere input aids vertical walking behaviour

Do haltere movements during walking provide useful input to the nervous system, or are they an epiphenomenon associated with locomotion? We ablated both halteres of *Sarcophaga* and *Drosophila* and observed walking behaviour. We did not observe any differences in walking on a horizontal surface, but noted that haltere-ablated *Sarcophaga* were less likely to exhibit the negative geotaxis (climbing a vertical wall) observed in their intact counterparts (figure 2c; Fisher's exact test,  $p \ll 0.001$ ). Similarly, a significantly higher proportion of haltere-ablated *Sarcophaga* fell off of the wall when gently

perturbed as compared to intact flies (Fisher's exact test,  $p \ll 0.001$ ). No differences were observed between intact and haltere-ablated *Drosophila* (which only moves the halteres in flight) in climbing or falling (figure 2c). These results suggest that haltere input is behaviourally relevant in behavioural contexts experienced during the fly's natural life.

### 3. Material and methods

#### (a) Animals

Flies were collected in Ohio, USA. *D. melanogaster* and *S. bullata* were taken from laboratory colonies. All flies were identified to family level using a dichotomous key [18].

#### (b) Flying flies

Flies were glued to pins and filmed in flight at 4000 frames  $s^{-1}$  (Fastec Imaging, San Diego, CA). The positions of the leading edge of the wing and haltere tip, as well as wing and haltere bases, were digitized using DLTDDataViewer [19]. We calculated phase of each haltere stroke relative to the wing stroke, as well as vector strength (see the electronic supplementary material).

#### (c) Walking flies

Flies walked freely in front of a camera capturing 2000 frames  $s^{-1}$ . Wings were removed to prevent flight and ensure haltere visibility. Positions of the haltere tip and base were digitized. In some trials, we observed both halteres using two synchronized cameras. We surveyed 23 fly families (figure 1a).

#### (d) Perturbation experiments

We glued a glass slide to a small vibration motor and applied a thin layer of Tanglefoot (Contech Inc., Vancouver, Canada) to the

slide. We placed all tarsi of a cold-anesthetized fly (*Sarcophaga* or *Drosophila*) onto the slide. When the fly assumed a normal posture, the platform was gently vibrated for 0.5 s via microcontroller (Arduino Uno, Sparkfun Electronics, Niwot, CO, USA). Responses were filmed and digitized as above.

#### (e) Electrophysiological recordings from indirect flight muscles during walking and flight

*Sarcophaga bullata* were tethered to a pin and implanted with silver wires (one recording and one ground) in the indirect flight muscles. See the electronic supplementary material for details.

#### (f) Vertical walking behaviour

Six individuals of *Sarcophaga* or *Drosophila* were simultaneously placed in a small plastic cup (Reditainer, 5.5 oz) and permitted to walk freely. The number of flies that climbed the wall of the cup was scored. The cup was then manually lifted a small distance (6.35 mm for *Sarcophaga*, 2 mm for *Drosophila*) and gently dropped. The number of flies that fell off the wall was scored for 22 repetitions of the experiment.

**Ethics.** Work complied with local ethical requirements.

**Data accessibility.** Data are available on Dryad: <http://dx.doi.org/10.5061/dryad.0s1q>.

**Authors' contributions.** J.L.F. and A.M.Y. designed experiments. J.M.H., D.P.M., A.M.Y., N.D.K. and S.M. collected data. J.L.F. wrote the manuscript with input from all authors. All authors approved the final version of the paper and are accountable for its contents.

**Competing interests.** We declare that we have no competing interests.

**Funding.** This work was funded by AFOSR grants nos. FA2386-13-1-3007 and FA9550-14-0398.

**Acknowledgements.** We thank Mark Willis and Cole Gilbert for valuable feedback, and Nicole Arnold for experimental assistance.

### References

- Dickinson MH. 1999 Haltere-mediated equilibrium reflexes of the fruit fly, *Drosophila melanogaster*. *Phil. Trans. R. Soc. Lond. B* **354**, 903–916. (doi:10.1098/rstb.1999.0442)
- Nalbach G. 1993 The halteres of the blowfly *Calliphora*. *J. Comp. Physiol. A* **173**, 293–300. (doi:10.1007/BF00212693)
- De Navas LF, Garaulet DL, Sánchez-Herrero E. 2006 The *ultrabithorax* hox gene of *Drosophila* controls haltere size by regulating the Dpp pathway. *Development* **133**, 4495–4506. (doi:10.1242/dev.02609)
- Fox JL, Daniel TL. 2008 A neural basis for gyroscopic force measurement in the halteres of *Holorusia*. *J. Comp. Physiol. A* **194**, 887–897. (doi:10.1007/s00359-008-0361-z)
- Brodsky AK. 1994 *The evolution of insect flight*. Oxford, UK: Oxford University Press.
- Deora T, Singh AK, Sane SP. 2015 Biomechanical basis of wing and haltere coordination in flies. *Proc. Natl Acad. Sci. USA* **112**, 1481–1486. (doi:10.1073/pnas.1412279112)
- Dickinson MH. 1990 Linear and nonlinear encoding properties of an identified mechanoreceptor on the fly wing measured with mechanical noise stimuli. *J. Exp. Biol.* **151**, 219–244.
- Chan WP, Dickinson MH. 1996 Position-specific central projections of mechanosensory neurons on the haltere of the blow fly, *Calliphora vicina*. *J. Comp. Neurol.* **369**, 405–418. (doi:10.1002/(SICI)1096-9861(19960603)369:3<405::AID-CNE6>3.0.CO;2-9)
- Pringle JWS. 1948 The gyroscopic mechanism of the halteres of Diptera. *Phil. Trans. R. Soc. Lond. B* **233**, 347–384. (doi:10.1098/rstb.1948.0007)
- Fayyazuddin A, Dickinson MH. 1999 Convergent mechanosensory input structures the firing phase of a steering motor neuron in the blowfly, *Calliphora*. *J. Neurophysiol.* **82**, 1916–1926.
- Fraenkel G. 1939 The function of the halteres of flies (Diptera). *Proc. Zool. Soc. Lond.* **A109**, 69–78. (doi:10.1111/j.1096-3642.1939.tb00049.x)
- Sandeman DC, Markl H. 1980 Head movements in flies (*Calliphora*) produced by deflexion of the halteres. *J. Exp. Biol.* **85**, 43–60.
- Miller PL. 1977 Haltere activity in a flightless hippoboscids fly, *Crataerina pallida*. *J. Insect Physiol.* **23**, 855–860. (doi:10.1016/0022-1910(77)90010-5)
- Wiegmann BM *et al.* 2011 Episodic radiations in the fly tree of life. *Proc. Natl Acad. Sci. USA* **108**, 5690–5695. (doi:10.1073/pnas.1012675108)
- Greene E, Orsak LJ, Whitman DW. 1987 A tephritid fly mimics the territorial displays of its jumping spider predators. *Science* **236**, 310–312. (doi:10.1126/science.236.4799.310)
- Fox JL, Fairhall AL, Daniel TL. 2010 Encoding properties of haltere neurons enable motion feature detection in a biological gyroscope. *Proc. Natl Acad. Sci. USA* **107**, 3840–3845. (doi:10.1073/pnas.0912548107)
- Chan WP, Prete F, Dickinson MH. 1998 Visual input to the efferent control system of a fly's 'gyroscope'. *Science* **280**, 289–292. (doi:10.1126/science.280.5361.289)
- Borror DJ, Triplehorn CA, Johnson NF. 1992 *An introduction to the study of insects*. Montréal, Canada: Saunders College.
- Hedrick TL. 2008 Software techniques for two- and three-dimensional kinematic measurements of biological and biomimetic systems. *Bioinspiration Biomimetics* **3**, 34001. (doi:10.1088/1748-3182/3/3/034001)