

UNIVERSITY OF OXFORD

The Biomechanics of the Dipteran Wing Hinge

A THESIS PRESENTED FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

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December 2018

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Presented for the Degree of **Doctor of Philosophy** in

Trinity Term 2018

Abstract

Dipteran insects include some of the most aerobatic animals on the planet, including species such as drone flies (*Eristalis tenax*) which are able to hold a steady hover in the face of environmental perturbations before executing rapid and precise manoeuvres. A deep understanding of the mechanisms of wing kinematic control that afford such abilities is vital if we are to emulate these capabilities in our own bio-inspired machines. This thesis makes use of cutting-edge techniques to visualise these mechanisms in unparalleled detail and determine how they function to control the way in which the wings move. My work here begins by documenting the mechanical interaction of two exoskeletal components around the wing hinge that forms the gear change mechanism of dipteran wing kinematic control. I show its effects on the movement of the wings and explain how the mechanism acts to bring about these changes in *Calliphora vicina* and *E. tenax*. I then move on to study another enigmatic structure near the wing hinge, the Pterale C, and argue against a role of this component in mechanically altering wing movement. Instead, I conclude that the Pterale C may be a sensory structure which informs the insect's central nervous system of the position and speed of the wing during the latter half of the downstroke. Finally, I utilise time-resolved synchrotron X-ray computed microtomography to visualise the internal musculature of *C. vicina* during flight, where I assert that several muscles influence wing movements by restricting the motion of wing hinge components. Throughout this thesis, the inhibition of wing motion and energy absorption pervades each mechanism. Through the application of our understanding of dipteran flight, it may be possible to design bio-inspired machines which begin to approach the aerial capabilities of these insects.

Acknowledgements

None of this work would have been possible without the help and support of my supervisors, Simon Walker and Graham Taylor. Their guidance and teaching over the course of my DPhil have enabled me to acquire skills as a researcher which will be invaluable in any career that I pursue in the future.

I am also very grateful for the guidance provided by Prof. Adrian Thomas and Prof. Fritz Vollrath during both my transfer and confirmation of status meetings. Their comments have helped to shape this thesis into its current form, and reminded me to draw my attention to the bigger picture and practical applications of this work.

My time at the synchrotron beamline at the Paul Scherrer Institut was made also far easier by the expertise of the incredibly knowledgeable technicians and researchers there. Most notably Dr. Rajmund Mokso and Dr. Christian Schlepütz. Dr. Schlepütz was also kind enough to offer his time to give advice and comments on the methods section of Chapter 7, for which I am extremely thankful. I also thank Alex Greenhalgh for his help in producing SEM images which have proved infinitely useful in illustrating various points across my thesis.

Heather Green in her role as Graduate Administrator has made the many administrative tasks required throughout the production of this thesis far easier than they would otherwise have been. I thank her for her help in this respect, and by being available to listen and help with any aspect of life in the department, she has made being a student in the Zoology department much better and enjoyable for both myself and every other Zoology postgraduate.

I also thank my mother, sister, father, and grandparents for their unwavering support and patience throughout both the highs and lows across the production of my thesis. My mother in particular has bore the brunt of my grievances, but has without fail managed to improve my mood and help me find a solution to any issue I've been faced with.

My friends in OURC have also made life in Oxford (and Bisley) much more enjoyable, helping me both to celebrate and to find the comic side of research when I most needed it.

Sabrina Martin was also one of my earliest friends in Oxford, and both she and her husband Sam Drury have become two of the closest friends Jen and I have had over the past four years. Regular trips to the pub, or dinner and drinks at their flat have proved to be the perfect answer to whatever question I've asked over my DPhil.

Finally, nobody has seen more of the best and worst moments over the past few years than my partner, Jen. Having met shortly after I arrived in Oxford and began my research, I can't say what life as a DPhil student in Oxford would have been like without her, but I'm certain it would have been far less enjoyable.

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0.1 List of Supplementary Videos

During this thesis I make reference to a number of supplementary videos obtained during my research to help explain and support my arguments. These videos can be accessed and downloaded through the following link:

https://github.com/JonnyWPage/Thesis_Videos

Supplementary video 3.1 - A magnified image of a *Calliphora vicina* wing hinge, highlighting various components in colour throughout the wingbeat. The video begins at the start of the downstroke, and the radial stop and pleural wing process engage in a gear 2 interaction towards the end of the downstroke. Radial stop - blue; Radial vein - green; Pleural wing process - red; Pterale C - yellow; anterior is to the right.

Supplementary video 3.2 - A magnified image of a *C. vicina* wing hinge, illustrating a gear 1 *RS-PWP* interaction. The region of interest is highlighted in a brightened area. Anterior is to the right.

Supplementary video 3.3 - A magnified image of a *C. vicina* wing hinge, illustrating a gear 2 *RS-PWP* interaction. The region of interest is highlighted in a brightened area. Anterior is to the right.

Supplementary video 3.4 - A magnified image of a *C. vicina* wing hinge, illustrating a gear 3 *RS-PWP* interaction. The region of interest is highlighted in a brightened area. Anterior is to the right.

Supplementary video 3.5 - A magnified image of a *C. vicina* wing hinge, illustrating a gear 2 *RS-PWP* interaction at the beginning of the upstroke. The region of interest is highlighted in a brightened area. Anterior is to the right.

Supplementary video 4.1 - A magnified image of a *Eristalis tenax* wing hinge, illustrating a gear 1 *RS-PWP* interaction at the beginning of the upstroke. The region of interest is highlighted in a brightened area and certain components are highlighted in colour at the start of the video. Radial stop - blue; Radial vein - green; Pleural wing process - red; Pterale C - yellow; anterior is to the right.

Supplementary video 4.2 - A magnified image of a *Eristalis tenax* wing hinge, illustrating a gear 1 *RS-PWP* interaction at the beginning of the upstroke. The region of interest is highlighted in a brightened area and certain components are highlighted in colour at the start of the video. Radial stop - blue; Radial vein - green; Pleural wing process - red; Pterale C - yellow; anterior is to the right.

Supplementary video 6.1 - A magnified image of a *C. vicina* wing hinge, illustrating *RS-PtC* interaction, and movement of the *PtC* independently of the surrounding wing hinge. The region of interest is highlighted in a brightened area.

Supplementary video 7.1 - A series of time-resolved μ CT images demonstrating the kinematics of the *I1* (red) and *I2* (green) muscle-tendon complexes. The surrounding area of the thorax has been removed to visualise these muscles from an interior viewpoint (i.e. looking towards the wing hinge from the inside of the insect). The right-hand wing of the insect can also be seen in the background. Note the tendon buckling exhibited by both muscles at the start of the downstroke and end of the upstroke.

Chapter 1

Introduction

Nature has been a constant source of both inspiration and envy across human culture and history, and nowhere is this more the case than in the pursuit of flight. Birds, bats and insects make flight look effortless, and yet until relatively recently in human history powered flight has been beyond our grasp. With advances in technology, materials, and our understanding of the aerodynamics of flight, however, we have developed machines that enable us to fly at speeds and scales not found in nature.

Modern materials and computing technologies now allow us to miniaturise our flying machines and design fully automated vehicles which can travel without the need for an on-board or even remote human pilot. However, with these advances we have encountered new challenges in designing and controlling these micro- and nano-unmanned air vehicles (M/NAVs). To find solutions to these problems we can once again look to the natural world for inspiration and an-

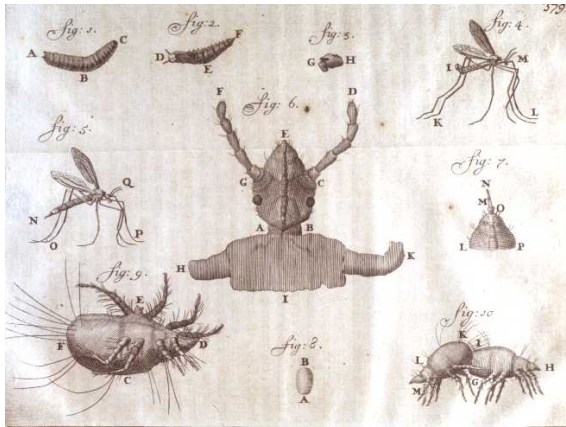


Figure 1.1: Technical drawings of several mites, insects and larvae by Antoni van Leeuwenhoek (1632-1723) using microscopy. Taken from his *Arcaana Naturae Detecta* (van Leeuwenhoek, 1695).

swers; and in the case of machines of such a small size, the aerobatic feats performed by insects are the ideal exemplar (e.g. Ellington (1999); Shyy et al. (1999); Wood

(2008); Sreetharan and Wood (2010); Floreano et al. (2010); Ramananarivo et al. (2011); Chin and Lau (2013); Ma et al. (2013); Elzinga et al. (2014); Harne and Wang (2015); Liu et al. (2016, 2017); Chen et al. (2017); Tang et al. (2017)).

In order to emulate what we see in nature, however, we require a deep understanding of it, and our knowledge of the mechanisms underlying the aerial capabilities of insects is as yet incomplete. Despite insects having fascinated scientists and artists for centuries (figure 1.1; van Leeuwenhoek (1695); van Kessel I (1626-1679); Smith and Coyle (2000); Green et al. (2007); Leather (2015)), our understanding of the biomechanics of flight motor that drives their aerial movement has lagged behind our knowledge of the flight of vertebrates. The anatomy of birds and bats can be studied without a microscope, and because they share the same body plan as all other tetrapod vertebrates, it is easier to gain an intuitive understanding of the biomechanics that control the movements of the various flight surfaces of these animals. Insects, on the other hand, possess none of these qualities. Some species of fairyflies (order: Mymaridae), for example, have body lengths of less than 300 μm (Makarova and Polilov, 2013; Polilov, 2017), therefore being smaller than some single-celled organisms, while insects such as mosquitoes (order: Diptera) exhibit wingbeat frequencies of around 1,000 Hz (Ogawa and Kanda, 1986; Bomphrey et al., 2018). Studying animals with either of these qualities is difficult, and combined these features make studying the biomechanics of these animals an extremely challenging pursuit.

This is not to say that the methods and techniques available have left us with a dearth of knowledge regarding insect flight. Both the internal and external anatomy of insects has been studied and documented extensively, providing us with a wealth of understanding, nomenclature, and terminology with which we can describe their biomechanics (e.g. Hollick (1940); Williams and Williams (1943); Pringle (1949); Roeder (1951); Nachtigall and Wilson (1967); Pringle (1981); Wisser and Nachtigall (1984); Gnatzy et al. (1987); Keil (1997); Deora et al. (2017)). Stroboscopic illumination, for example, subverts the inability of the human eye to visualise the

movement of components at the rapid speeds exhibited by insects, and in doing so it has revealed unexpected interactions between components of the hinge that connects the wing to the body (Wisser, 1988; Nalbach, 1989). We have also been able to gain some appreciation of how muscular activity influences the movement of the wing because of intricate electrophysiological studies and experiments (e.g. Nachtigall and Wilson (1967); Kutsch and Hug (1981); Heide (1983); Egelhaaf (1989); Dickinson et al. (1993); Tu and Dickinson (1996); Lehmann and Götz (1996); Heide and Götz (1996); Balint and Dickinson (2004); Bartussek et al. (2013)). These techniques have proved fruitful, but there are limits to the information we can glean from their use. There have been extreme difficulties in determining how exactly interactions between exoskeletal components in the wing hinge result in movements of the wing, and how the muscles within the flight motor influence this (Boettiger and Furshpan, 1952; Miyan and Ewing, 1985a; Wisser, 1987; Ennos, 1987; Pfau, 1987; Miyan and Ewing, 1988). Many attempts have been made to do this, but the use of unsuitable methods has sometimes led to highly criticised conclusions. The use of carbon tetrachloride as an anaesthetic by some authors to study the movement of wing hinge components in dipteran insects (Boettiger and Furshpan, 1950, 1952), for example, is now thought to have caused unnatural movements and orientations of these components due to muscle tetanus induced by the chemical compound (Miyan and Ewing, 1985b).

Fortunately, novel technologies now allow us to visualise these animals in ways that were previously impossible. With this, our potential for understanding the insect flight motor, and for emulating it, has increased markedly. Developments in high-speed cameras, for example, now allow us to film insects at both high frames rates and rapid shutter speeds, and have been used to film the detailed movements of insect wings during flight. Photogrammetry techniques can then also be applied to resolve the position of the wing, and even its shape, in three dimensions (figure 1.2). In doing so, properties of the wing have been identified which aid in the production of aerodynamic force during the wingbeat, such as its ability to twist

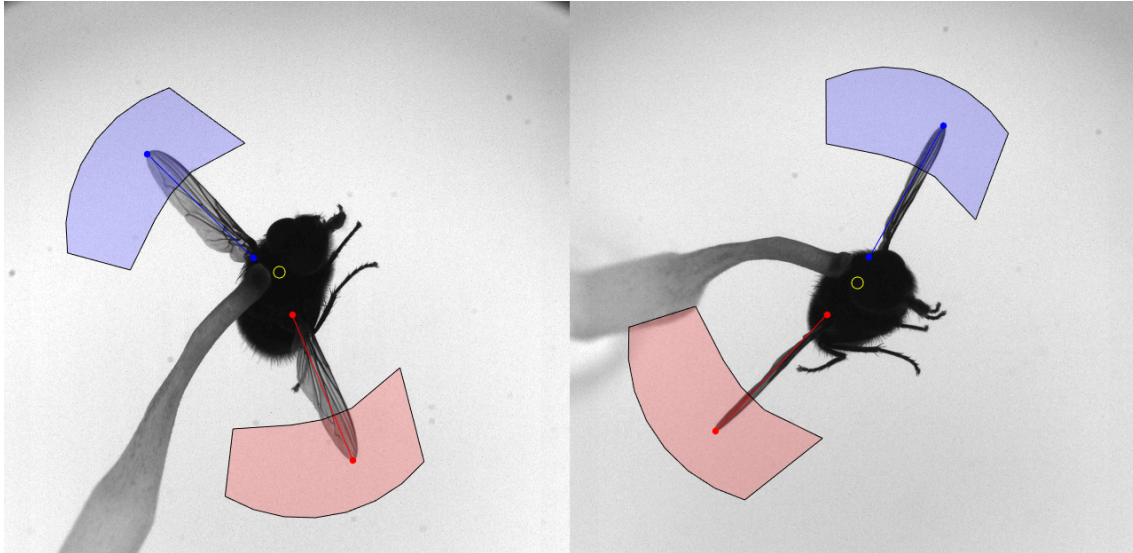


Figure 1.2: Images taken from two high-speed video recordings of tethered *Calliphora vicina* flight, demonstrating the three-dimensional photogrammetric tracking of the wingtip positions and wing length over the wingbeat cycle.

or camber, and the intricacies of these movements in relation to the direction of the wing's motion (Wolf, 1993; Walker et al., 2009, 2010, 2012; Risse et al., 2013). Using other techniques, such as particle image velocimetry, these different wing properties and movements can further be evaluated in terms of their effects on the aerodynamic force output of the wing (Ellington, 1984a; Dickinson et al., 1999; Sane and Dickinson, 2001; Fry et al., 2003; Balint and Dickinson, 2004; Thomas et al., 2004; Bomphrey et al., 2005; Bergou et al., 2010; Muijres et al., 2014). Furthermore, these same cameras can now also be used to develop detailed, natural-colour 3-D models of the insect's entire body (Nguyen et al., 2016) to examine how the various components of the thorax articulate with each other.

When applied to macrophotography, high speed cameras can also allow us to monitor the movement of the small, rapidly moving structures of the wing hinge. This has afforded us a deeper understanding of previously identified interactions between these elements (e.g. Deora et al. (2015)), and will continue to help us develop mechanistic models that describe how the movement of each element translates into wing motion.

A full model of the wing hinge will also include the properties of the muscles that attach to these wing hinge components, including their activity patterns, and mech-

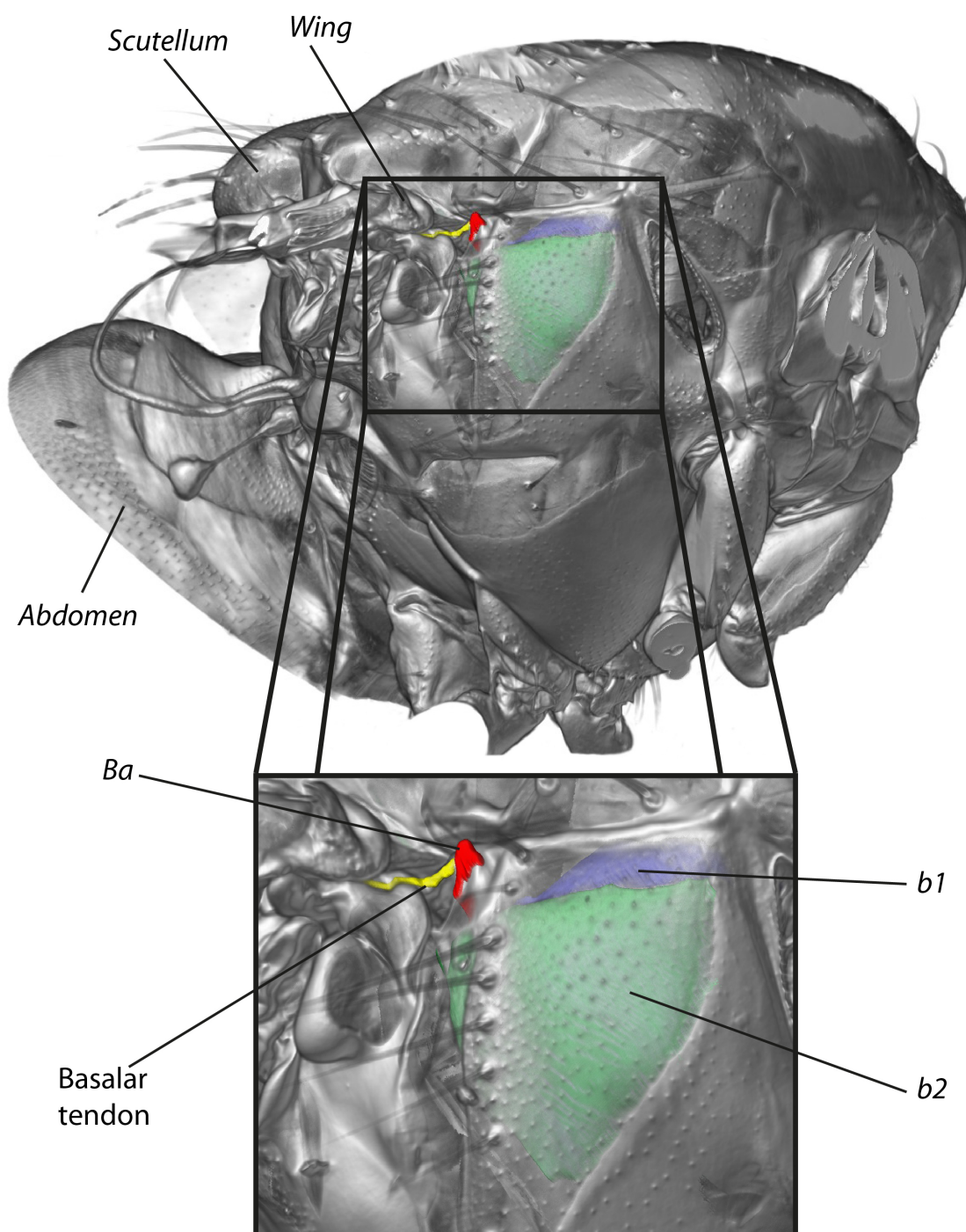


Figure 1.3: An image of the thorax and upper abdomen of *Calliphora vicina* reconstructed using synchrotron X-ray computed microtomography, with some components of the wing hinge (and their associated muscles) highlighted in colour. Abbreviations: *Ba* - basalar sclerite; *b1* - first basalar muscle; *b2* - second basalar muscle.

anisms of action. Our knowledge of muscular activity patterns has been deepened with the developments in genetic engineering, and when combined with photogrammetry this technique can uncover detailed relationships between muscular activity patterns and wing kinematics (Lindsay et al., 2017). Using other cutting-edge techniques such as time-resolved computed microtomography it is now even possible to visualise the movement of this flight musculature during flight. This method allows us to observe the movements of both the muscles and the components to which they attach, as well as determine the mechanisms through which these muscles bring about changes in wing kinematics (figure 1.3; Walker et al. (2014)).

Throughout my work presented here, I have made use of several modern techniques to answer some of the many questions that remain regarding the biomechanics of the dipteran flight motor. This thesis begins with a literature review, outlining our current knowledge of the flight motor across Diptera. The following two chapters use high-speed videography and photogrammetry to examine the gear change mechanism in the blow fly, *Calliphora vicina*, and drone fly, *Eristalis tenax*, determining the effects of this mechanism on wing movement. I also identify significant differences in the mechanism between these two species, relating these to differences in their anatomies, and using these findings to infer how the mechanism functions. The next chapter continues this discussion by performing ablation experiments and examining the movement of wing hinge components in more detail. After this, I move on to discuss the role of a poorly understood part of the wing hinge, the Pterale C, and identify how this organ may aid the fine control of wing kinematics. The final chapter switches focus from the flight motor's external anatomy, to the internal musculature that attach to these wing hinge components. Using time-resolved synchrotron X-ray computed microtomography, we visualise the movement of several muscles within the flight motor, and infer mechanisms through which they may act to determine wing kinematics. I then conclude my work with a synthesis of my findings across these chapters.

Throughout this thesis, I draw attention to how my various findings may be used

to improve the efficacy and efficiency of micro- and nano-machines, highlighting underutilised engineering principles which may prove more effective than current paradigms. The work I present here represents significant steps forward not only in our understanding of the flight of these animals, but also in our capacity to apply this knowledge to solve the myriad problems that engineers face when designing machines with the capability to match these insects in flight.

Chapter 2

Literature Review: A

Tomographic Illustration of the Flight Motor

Just like volant vertebrates, insects make use of muscles to control their wing movements during flight. However, whereas these muscles attach to and within the wings in vertebrates, neopteran insects such as flies (order Diptera) have no muscles connected directly to the wings, and as such the mechanisms through which these animals control their wing movements are completely different. Dissection and electrophysiology have provided a wealth of knowledge regarding muscular activity, and kinematic analysis has revealed interactions between the wing and other parts of the exoskeleton. However, methodological limitations have meant that our knowledge of the biomechanics of these muscles and exoskeletal interactions has lagged behind our understanding of the flight motor's physiology. Fortunately, novel methods such as time-resolved synchrotron X-ray computed microtomography are enabling us to visualise the insect flight motor in unparalleled detail, and are revealing the mechanisms through which the wings are controlled. This review summarises our current understanding of wing kinematic control in the context of the dipteran flight motor. We discuss the flight control musculature, the gear change mechanism, and

the possible role of a prominent but poorly understood component, the Pterale C. Across these elements of the flight motor, a common function is the restriction of movement of other components through the absorption of energy or the inhibition of flight motor component motion. We suggest that the wings are primarily controlled through the use of energy absorption or negative work, and highlight techniques which will identify whether this is indeed the case. Improving our understanding of the dipteran flight motor will improve our ability to apply the engineering principles found within these insects to the design of more efficient and effective micro- or nano-air vehicles.

2.1 Introduction

Powered flight has evolved in only four groups of animals: insects, birds, bats and pterosaurs, but these groups are extremely diverse (Mora et al., 2011). It is no coincidence that these animals are also some of the most speciose, as flight allows animals to explore a three-dimensional space not fully accessible to terrestrial animals. Flying species are often able to forage, hunt, migrate and evade predation more effectively than terrestrial animals despite the significant energetic costs of flight (Ydenberg and Dill, 1986; Obst and Nagy, 1992; Maina, 2000; Nudds and Bryant, 2000; Voigt and Speakman, 2007; McKay et al., 2016).

The benefits of aerial locomotion have not gone unnoticed by engineers, who have successfully designed a huge number of vehicles with flight capability. However, the development of flying machines at small scales similar to birds or insects (i.e. operating at low Reynolds numbers) has proved particularly challenging. Engineers are therefore increasingly beginning apply to the solutions found in nature to the difficulties encountered during the design of these micro air vehicles (MAVs; e.g. Ellington (1999); Shyy et al. (1999); Ramanananarivo et al. (2011); Chin and Lau (2013); Liu et al. (2017); Chen et al. (2017)).

In order to take advantage of this bio-inspired methodology we first require a deep understanding of the biological system in question. The flight of birds has

been extensively studied, meaning we have a detailed knowledge of the mechanical function of the anatomy of these animals (e.g. Tobalske (2000); Taylor et al. (2003); Altshuler and Dudley (2006); Tobalske (2007); Clark (2009)). Insects, on the other hand, have proved more difficult to study and understand due to their small size, complex anatomy and internalised musculature. Despite these obstacles, extensive studies have been performed using traditional techniques such as dissection, electrophysiology, stroboscopic illumination, while more modern techniques such as high speed photography and photogrammetry have provided a wealth of knowledge regarding the biomechanics of flight in insects (e.g. Jensen and Weis-Fogh (1962); Heide (1983); Wisser and Nachtigall (1984); Nalbach (1989); Dickinson and Tu (1997); Walker et al. (2009)).

Flying insects (subclass Pterygota) are split into two subdivisions: the Palaeoptera and Neoptera, which differ in the mechanisms through which they produce wing movements. The Palaeoptera, which includes dragonflies and damselflies (Order Odonata), mayflies (Ephemeroptera) and the extinct Palaeodictyopterida, are often characterised by the fact that they cannot fold their wings over their body (Hovmöller et al., 2002; Willkommen and Hörnschemeyer, 2007; Thomas et al., 2013). In these insects, two large pairs of muscles attach directly to the wings, the elevator and depressor muscles, which are responsible for raising and lowering the wings, respectively, to produce a wingbeat (Chapman, 2013). Like vertebrate skeletal muscles, these palaeopteran flight muscles respond directly to nervous innervation, producing a contraction in response to an action potential in a one-to-one fashion (Peckham and White, 1991). They are therefore termed ‘synchronous’ muscles, and are responsible for controlling both the wingbeat and altering wing kinematics during flight.

Neopteran insects, on the other hand, have no muscles attaching directly to the wings. Instead, two large pairs of antagonistic muscles, the dorsolongitudinal muscle and dorsoventral muscles (DLM and DVMs, respectively, or the indirect power muscles collectively), work to deform the thorax, and in doing so induce move-

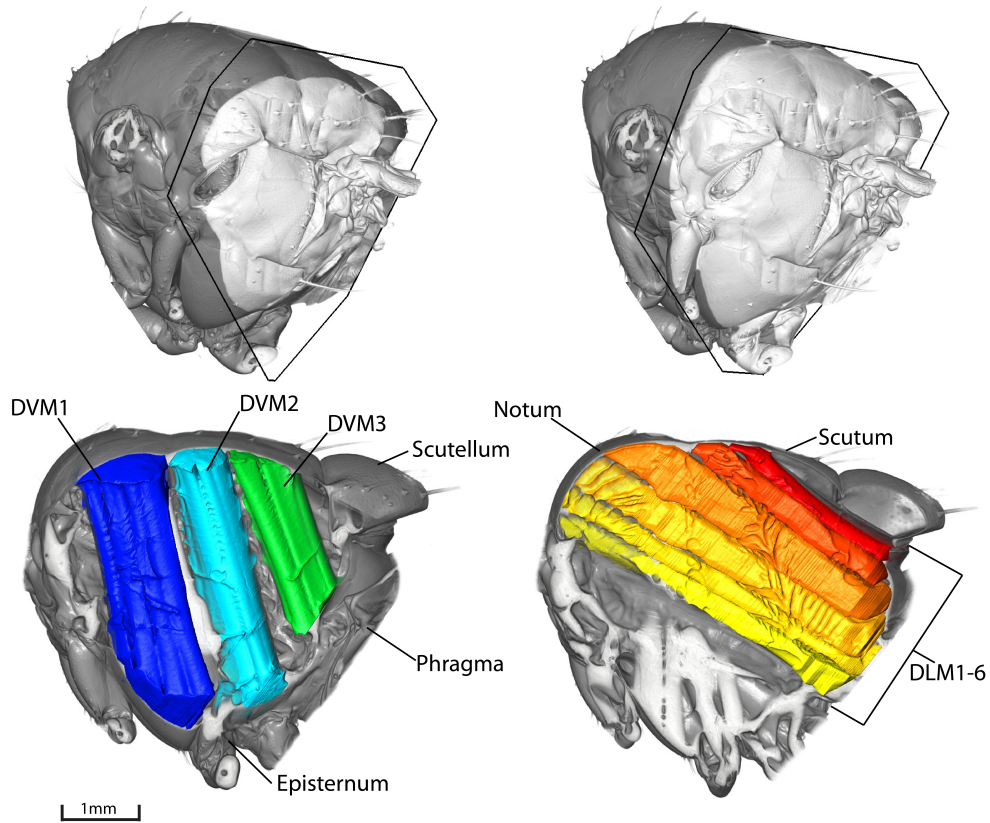


Figure 2.1: μ CT scans of the thorax of *Calliphora vicina*, windowed to reveal the anatomy of the power muscles (colour) and the parts of the thorax to which they attach (grey). Top: Figures illustrating the plane through which the thorax has been windowed (the white area indicates the volume which has been removed). Bottom left: The three dorsoventral muscles (DVMs). Bottom right: The six subunits of the dorsolongitudinal muscle (DLM). Scale bar = 1mm.

ments of the various exoskeletal components (sclerites) that make up the wing hinge (figure 2.1). These wing hinge sclerites articulate with the wing, and so their movement results in a wingbeat (Boettiger and Furshpan, 1952; Thomson and Thompson, 1977; Miyan and Ewing, 1985a; Bennet-Clark, 1986; Ennos, 1987; Pfau, 1987; Miyan and Ewing, 1988). In some neopteran orders (i.e. Diptera, Coleoptera, many of the Hemiptera, and some Hymenoptera) the DVMs and DLM are asynchronous, meaning that they do not receive nervous input during each contraction. Instead, these muscles exhibit delayed stretch activation and delayed shortening deactivation. These characteristics mean that stress within the muscle increases with a slight delay after lengthening begins, and the muscle remains active throughout the whole shortening cycle, before stress decreases at the beginning of the next lengthening cycle (Roeder, 1951; Machin and Pringle, 1960; McCann and Boettiger, 1961; Pringle,

1981; Josephson et al., 2000; Bartussek et al., 2013). As the DVMs and DLM act antagonistically to each other, when DVMs contract and cause the wings to beat through the upstroke, the DLM is stretched. The combination of delayed stretch activation and delayed shortening deactivation within these fibrillar muscles produces an oscillatory motion that then continues without the need for nervous input during each wingbeat (though occasional action potentials are received by the muscles to ensure adequate calcium concentrations for contraction). This stretch activation feature of the asynchronous muscle allows neopteran insects to produce extremely high wingbeat frequencies, and do so with a large amount of power: the calcium cycling normally required by synchronous muscles during contraction/relaxation cycles is not necessary, and the area which is normally occupied by the sarcoplasmic reticulum (in which calcium is stored within muscle cells and released upon nervous stimulation) can instead be replaced by more contractile myofibrils (Josephson et al., 2000).

Neither the DVMs or the DLM attach to the sclerites that make up the wing hinge, and so they are unable to bring about changes in the kinematics of the wings during flight. A set of synchronous muscles, termed the direct control muscles, do attach to these sclerites, and it is their role to influence the movement of the wing hinge components (figure 2.2). In doing so, these muscles determine the precise movements of the wings during the wingbeat, and are sometimes termed the steering muscles because of this. Another set of synchronous muscles, the indirect control muscles, attach elsewhere in the body and alter wing motion through changes in the properties of the thorax, such as its stiffness. As the control muscles are orders of magnitude smaller than the power muscles, they must make use of mechanisms to amplify their work output to have any significant impact on the motion of the sclerites, and do so at high frequencies. One such feature may be the reliance on negative work (resulting from eccentric muscle contraction), which allows muscles to generate more force and do so more efficiently when compared to positive work (Fenn, 1924; Abbott et al., 1951, 1952; Bigland-Ritchie and Woods, 1976; Lindstedt

et al., 2001; Elmer and LaStayo, 2014; Ferenczi et al., 2014). This is thought to be a result of the contribution of elements such as titin and attached crossbridges in generating tension during lengthening, and the reduced need for crossbridge cycling to generate force (Pinniger et al., 2006; Nishikawa et al., 2012; Nishikawa, 2016; Hessel and Nishikawa, 2017). This is a feature already identified in some of the control muscles (section 2.6; Tu and Dickinson (1994, 1996)), and that may be present in others too.

In changing the movement of the wing hinge sclerites, the control muscles are also thought to control wing movements by causing sclerite interactions. One of which, the gear change mechanism, is a mechanical interaction between two sclerites known as the radial stop (*RS*) and pleural wing process (*PWP*). This interaction and its effects have been studied by numerous different authors (Miyah and Ewing, 1985a; Pfau, 1987; Wisser, 1988; Nalbach, 1989; Balint and Dickinson, 2001; Walker et al., 2012; Deora et al., 2015), but different conclusions have been drawn regarding its precise function (section 2.4).

Studying interactions of wing hinge components also reveals the presence of another poorly understood component: the Pterale C (*PtC*). The *PtC* is found on the anterior edge of the wing hinge and contacts the *RS* during the latter half of the downstroke, but the significance of this interaction has received remarkably little attention. The prominence of the *PtC* within the wing hinge indicates its likely importance in wing kinematic control, either through sensory or mechanical means. Some authors who have studied this organ claim it is a proprioceptive organ (Miyah and Ewing, 1984, 1985a; Nalbach, 1989; Fayyazuddin and Dickinson, 1996), while others assert it instead acts as a shock absorber and slows the wing down as it approaches the end of the downstroke (Chowdhury and Parr, 1981; Wisser, 1988; Nalbach, 1989).

The various interactions of these components of the dipteran flight motor are complex, and there are still significant gaps in our knowledge of their effects on wing kinematics. Fortunately, modern techniques now enable us to visualise the

movement of the wings in previously impossible ways. High speed macrophotography allows us to visualise the movement of the miniscule components that make up the wing hinge, and photogrammetry techniques enable us to resolve the movement of the wings in three dimensional space. We can even visualise the movement of the internalised musculature of insects using time-resolved synchrotron X-ray computed microtomography (μ CT), which has opened up new possibilities for comprehending the remarkable complexities of the dipteran flight motor (Walker et al., 2014; Mokso et al., 2015).

This review describes the anatomy and biomechanics of the dipteran flight motor and considers what aspects are not fully understood as a motivation for the experimental work contained within this thesis. To illustrate the complex three-dimensional anatomy of the system, we make use of images of the flight motor collected through synchrotron X-ray computed microtomography (μ CT). We begin by briefly describing this technique, before moving on to review our current understanding of the biomechanics of wingbeat generation and control. Beginning at the generation of the gross wing movement that forms the wingbeat, we then examine the mechanisms of coarse wing kinematic control through the gear change mechanism and the potential role of the *PtC* in this context. This is then followed by a detailed anatomical description of the various control muscles within the thorax and the fine control over wing movement that they exert.

Finally, we conclude by highlighting a common theme running throughout this review: the absorption of energy and the inhibition of component movement. The gear change mechanism, *PtC*, and some control muscles (*b1* and *b2*) have all been suggested to shape wing kinematics by restricting wing and sclerite movement, thereby absorbing kinetic energy in the process. This review outlines the current understanding of these aspects of the dipteran flight motor, and reveals the possible extent to which dipteran insects (and other neopterans) rely on energy absorption and negative work to control their wing movements.

The conclusions of this work will be of interest to those working in the field of

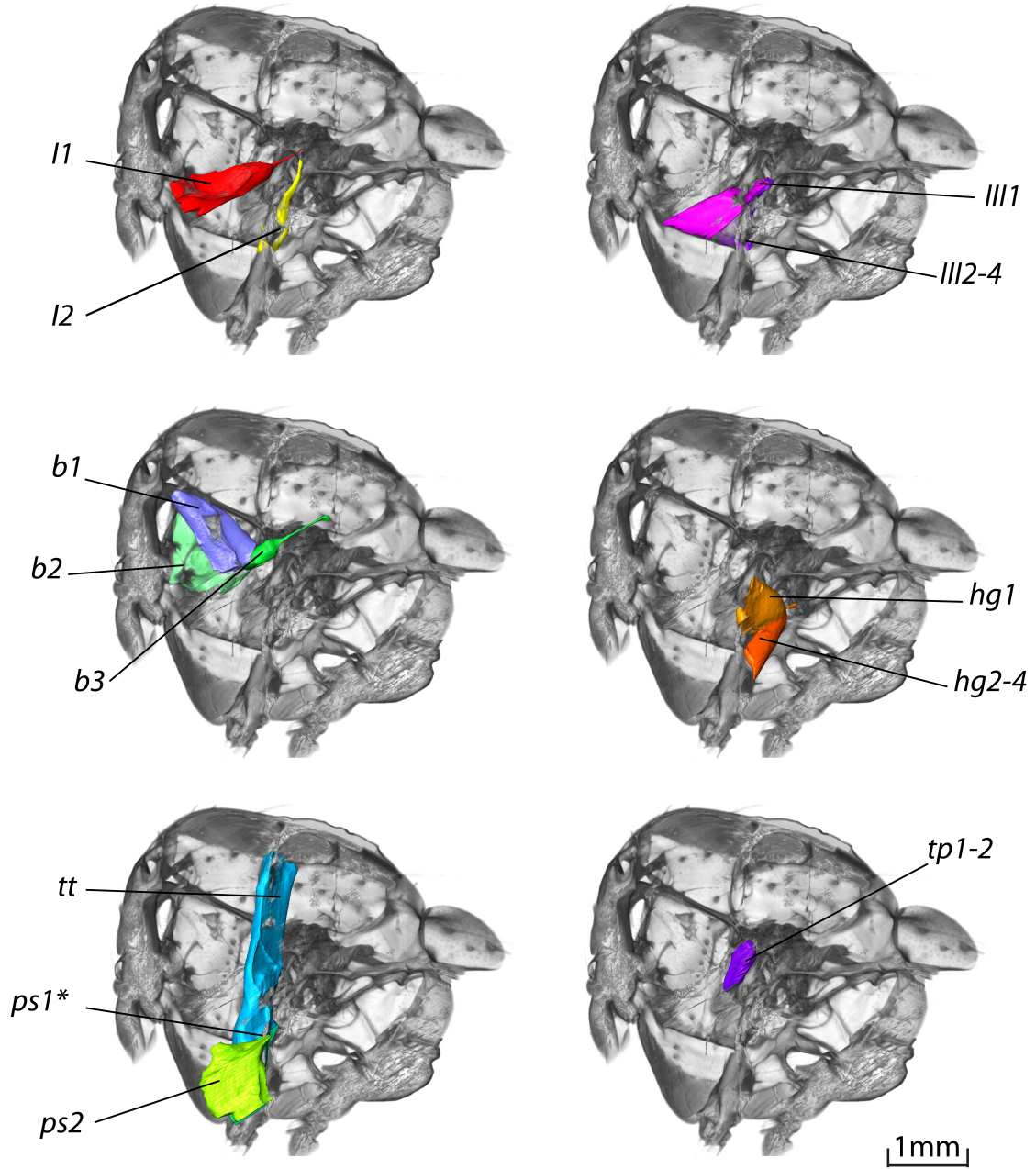


Figure 2.2: μ CT scan cross sections of the *C. vicina* thorax highlighting the various control muscles: the muscles of the first axillary sclerite (*I1* and *I2*), the third axillary sclerite (*III1* and *III2-4*), the basalar sclerite (*b1*, *b2*, and *b3*), the posterior notal wing process (*hg1* and *hg2-4*), the tergotrochanter (*tt*), the pleurosternal (*ps1* and *ps2*), and the tergopectoral muscles (*tp1* and *tp2*). *Note that most of *ps1* is obscured by *ps2* from this viewpoint. Scale bar = 1mm.

arthropod biomechanics, and also to those currently working to design bio-inspired vehicles or other systems. By inspecting the biomechanics underlying the flight capability of these remarkable insects, this literature review may highlight novel mechanisms which can be adapted for use in artificial designs.

2.2 X-Ray μ CT imaging of the Flight Motor

Much of the difficulty in understanding the biomechanics of the dipteran flight motor stems from the miniscule size of the components involved, and the complex internalised musculature which is responsible for controlling the movement of these various parts. These problems can be partially managed through the employment of high-power microscopes and skilled dissection, but these methods are prone to subjectivity, unrepeatability, and error. It is also difficult to export the raw findings of these observations for corroboration by other workers in the field outside of pictures or videos of the dissection. While dedicated work in this field has produced a wealth of knowledge regarding the anatomy of these animals, attempts to understand their movement through the physical manipulation of the body has sometimes led to spurious results which have since been criticised. One such example is the ‘click’ mechanism of wing movement (Boettiger and Furshpan (1952); Thomson and Thompson (1977); Pfau (1987); Brennan et al. (2003); Tang and Brennan (2011) but see Miyan and Ewing (1985b)), an inaccurate mechanistic theory of the wing hinge which has been largely based on evidence acquired from the manipulation of insects anaesthetised with carbon tetrachloride. This analgesic is known to induce tonic contraction of the pleurosternal muscles, which increases tension within the thorax and forces the wing hinge components into an unnatural position (Miyan and Ewing, 1985b). In this state, the wing can rapidly ‘click’ between an elevated and depressed position, and thereby lead to the erroneous conclusion that the wing hinge is a bistable structure.

Studying the movement of these insects when alive is unfortunately extremely difficult. Many dipteran insects beat their wings at over 150 Hz, and some, such as

mosquitoes, can reach wingbeat frequencies of nearly 1,000 Hz (Ogawa and Kanda, 1986; Bomphrey et al., 2018), making their movement impossible to visualise without the use of specialist equipment. High speed videography can allow us to film the movement of the exoskeleton, but this masks the motion of the internalised musculature. It is possible to perform ‘windowing’, by which a part of the exoskeletal cuticle is removed to reveal the muscles (e.g. Chan and Dickinson (1996)). However, since the exoskeleton itself is part of the flight motor, doing so will likely change the biomechanics of the system.

Fortunately, computed microtomographic (μ CT) imaging circumvents many of the issues inherent in dissection. This technique enables repeatable, non-destructive observations of many individuals, the data from which can be shared with any number of researchers with access to the appropriate open-source or commercial software. Furthermore, μ CT imaging enables the simultaneous collection of high-resolution images (on a μm scale) of both the internal and external anatomical features, so that the articulations between these components can be explored without the risk of damage to the specimen. Static μ CT imaging has become an increasingly popular technique in the study of the anatomy and biomechanics of arthropods (e.g. Hörnschemeyer et al. (2002); Liu et al. (2010); Li et al. (2011); Kim et al. (2012); Shaha et al. (2013); Iwan et al. (2015); David et al. (2016); Davranoglou et al. (2017); Deora et al. (2017); Wasserthal et al. (2018)), although most of these studies make use of dried or fixed specimens that are likely to exhibit preservation artefacts. Much faster imaging can be performed using synchrotron X-ray μ CT, which therefore enables the visualisation of fresh or living specimens. An extension of this technique, synchrotron X-ray time-resolved μ CT, allows us to visualise the anatomy of moving specimens, having first been applied to the flight of *C. vicina* (Chapter 7) & Walker et al. (2014); Mokso et al. (2015)). Here we make use of these techniques to produce high-resolution images of the flight motor of *C. vicina* at various stages during the wingbeat. These images are used to illustrate various anatomical features discussed in this review, and how they articulate with each other

to produce and control a wingbeat.

2.2.1 Collection of μ CT Images

We acquired time-resolved microtomographic images of twenty *C. vicina* individuals using the TOMCAT beamline at the Swiss Light Source facility (Paul Scherrer Institut, Villigen, Switzerland; Stampanoni et al. (2010); Walker et al. (2014)). An image of the experimental set up including high speed camera position and specimen location relative to the X-ray beamline and detector can be seen in figure 2.3. Details of methodology can be found in appendix A.1.

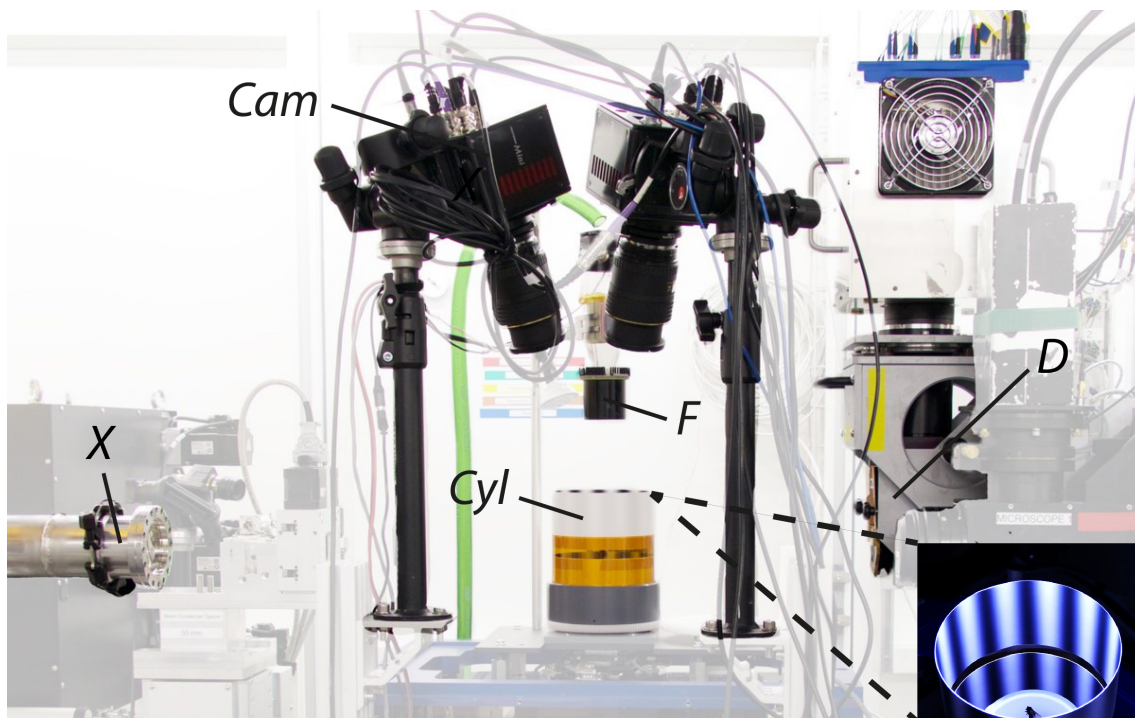


Figure 2.3: The experimental set up at the Swiss Light Source TOMCAT beamline. Parts of the image have been greyed out for clarity. *Cyl* - The patterned cylinder within which the insect and LED ring is positioned; orange polyimide Kapton® tape connects the cylinder's two halves and permits the movement of X-rays through to the insect's thorax. The pattern, insect and diffuser above the LED ring can be seen in the bottom right panel. This pattern was an experimental manipulation intended to induce fictive turning behaviour by the fly (see Chapter 7)). *Cam* - The configuration of two high-speed cameras filming the insect within the cylinder. *D* - The scintillator and GigaFRoST detector. *F* - The fan that directed a humidified airstream towards the insect.

2.3 Wingbeat Production and Control

To generate wing movement, all dipteran insects make use of two large (occupying $92.3 \pm 1\%$ of the total muscle volume within the thorax in ten of our specimens) paired sets of indirect power muscles within the thorax, which drive wing movement by deforming the thorax (figures 2.1 & 2.5). The three dorsoventral muscles (DVMs) run dorsoventrally within the thorax, attaching dorsally to the scutum and ventrally to the episternum, while the dorsolongitudinal muscle (DLM) runs obliquely down the long axis of the fly, attaching cranially to the notum and scutum, and caudally to the phragma (figures 2.1 & 2.4, and see Wisser and Nachtigall (1984) for a detailed description). As the DLM contracts during the downstroke, the thorax is compressed antero-posteriorly and expands dorsoventrally. This movement causes a ventral rotation of the scutellum about its joint with the scutum, and this force is then translated through the scutellar lever into ventrally directed wing movement (figures 2.4 & 2.5; Boettiger and Furshpan (1952); Thomson and Thompson (1977); Miyan and Ewing (1985a,b); Bennet-Clark (1986); Ennos (1987); Pfau (1987); Miyan and Ewing (1988); Brennan et al. (2003); Tang and Brennan (2011); Chapman (2013)). As the DVMs contract during the upstroke, the thorax is compressed dorsoventrally while it expands antero-posteriorly, resulting in a dorsal rotation of the scutellum. As this movement is translated through the scutellar lever, the wings are swung dorsally. The DLM and DVMs act antagonistically, and due to their stretch-activated nature as soon as one set is finished contracting, the other begins, resulting in a rapid wingbeat that requires only occasional nervous excitation (Pringle, 1949; Dickinson and Tu, 1997). This mechanism of producing high frequency movements has proved such an effective solution that it is thought to have evolved independently several times (Cullen, 1971; Pringle, 1981).

Due to its connection with other components of the thorax the scutellum predominantly rotates dorsoventrally, with very little mediolateral or transverse rotation. This in turn means that there is little scope for bilateral asymmetries in the force transmitted to the wing hinges, which is crucial for inducing manoeuvres. The total

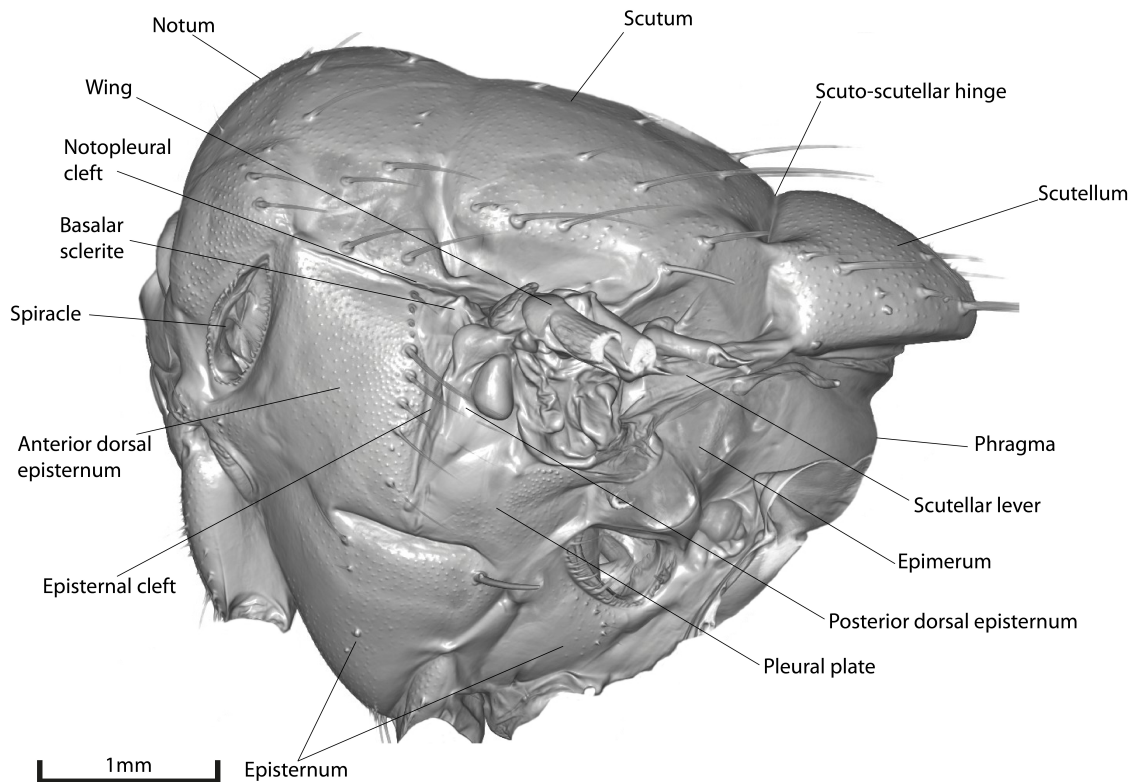


Figure 2.4: An annotated μ CT scan of a *C. vicina* thorax. Anterior is to the left, posterior is to the right. Scale bar = 1mm.

force of contraction from all the power muscles is summed together by the scutellum into a single, symmetrical and phase-locked motion. Indeed, if the scutellum is lesioned along the insect's body axis, the wings are able to move independently of each other (Deora et al., 2015). It should be noted that there has been some indication of small asymmetries in wing movement produced by bilateral asymmetries in calcium concentration within the power muscles of *Drosophila* (Lehmann et al., 2013). However, these bilateral asymmetries are not sufficient to bring about the rapid manoeuvres that these insects perform. Instead, such asymmetries in sarcoplasmic calcium level serve to trim the aerodynamic power output of the flight motor, possibly allowing the insect to compensate for asymmetric damage or development.

The control muscles, on the other hand, share no common linkages between the two sides of the body, and so are free to act independently (figure 2.2). These muscles are classed as one of two types: the 'direct' control muscles which attach to and influence the movement of the wing hinge sclerites, and the 'indirect' control muscles which attach elsewhere in the thorax, and act by altering the properties

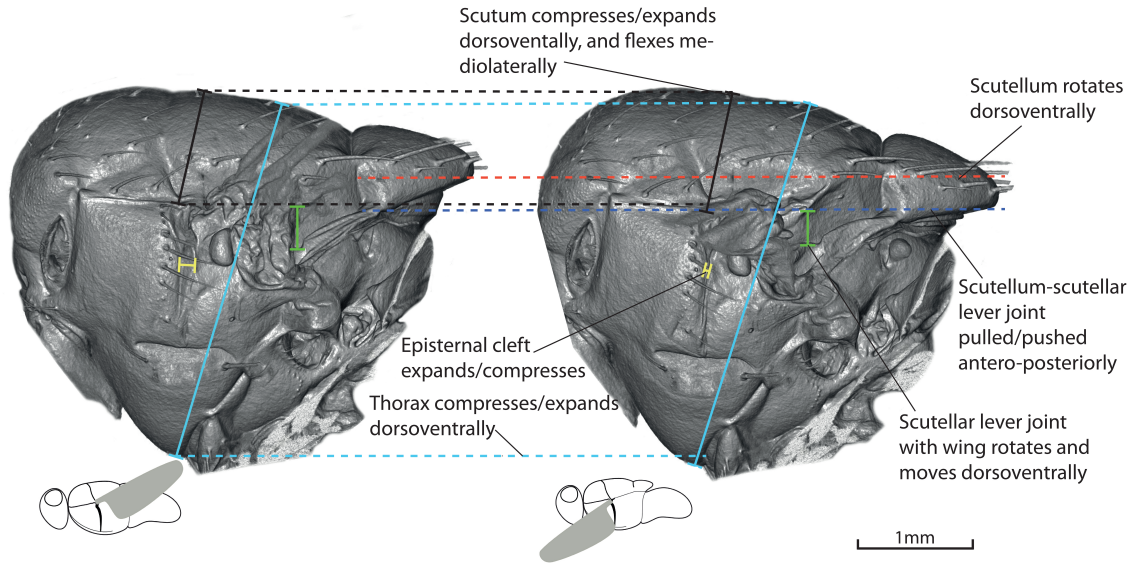


Figure 2.5: Annotated pCT scan images of a *C. vicina* thorax at the beginning (left) and end (right) of the downstroke, with the wing positions shown by the schematics below each image. Movements of various thorax components during the upstroke/downstroke are highlighted. Dashed lines highlight the differences between the position of the various components at the start of the upstroke compared to the end of the downstroke.

of the thorax itself (such as its stiffness and resonant properties; Pringle (1949); Roeder (1951); Greenewalt (1960); Dickinson and Lighton (1995); Brennan et al. (2003); Ha et al. (2013); Deora et al. (2017)). The wing movement induced by the power muscles is thereby altered by these very small muscles. In this way, the power muscles act as the engine: powering wing movement. The control muscles, on the other hand, act as the transmission: changing the way that power is translated into wing movement (Dickinson and Tu, 1997).

2.4 The Gear Change Mechanism

Dipteran flight control can be viewed as operating on two hierarchical levels. The first is a coarse ‘gear change’ mechanism involving discrete shifts in the engagement of the sclerites at the wing base, while the second is a series of subtler changes in muscle action which interact with the gear change mechanism to produce fine motor control.

The gear change mechanism of dipteran flight has been studied by a number of different authors (Pfau, 1973; Miyan and Ewing, 1985a; Pfau, 1987; Wisser, 1988;

Nalbach, 1989; Balint and Dickinson, 2001; Walker et al., 2012; Deora et al., 2015). The proposed mechanism involves the interaction of a structure known as the pleural wing process (*PWP*) with another called the radial stop (*RS*). The *PWP* consists of a highly sclerotised stalk that projects laterally from the dorsal area of the pleural plate of the thorax and ends with a bulbous head (figure 2.6). The precise shape of the head varies between species (Miyani and Ewing, 1984), but in *Calliphora* it is heart-shaped with three peaks along its dorsal edge separated by two grooves.

The *RS*, on the other hand, is an expansion of cuticle found ventrally on the proximal end of the second most anterior vein of the wing, the radial vein (Miyani and Ewing (1985a) see figures 2.6 & 2.9). During the wingbeat, the *RS* may fit into one of the grooves found on the *PWP* (figures 2.7, 2.6 & 2.9); or alternatively the two may not interact at all. Given that the interaction of the *RS* and *PWP* is thought to form a mechanism of coarse wing kinematic control about which finer details are changed, our understanding of it provides essential context for comprehending the complete operation of the dipteran flight motor. Unfortunately, the detailed operation of the gear change mechanism has been the subject of some controversy.

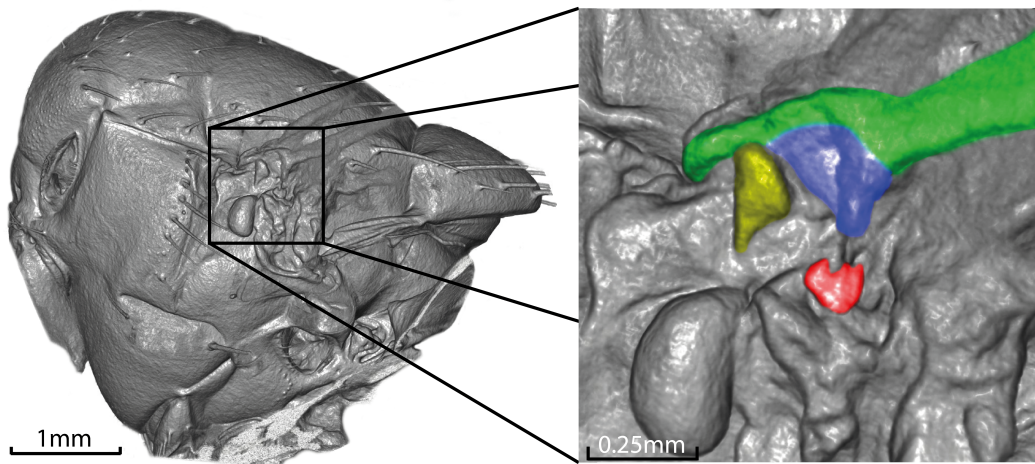


Figure 2.6: A μ CT scan image of the left side of a *C. vicina* thorax (anterior to the left, posterior to the right), magnified to display various important components of the wing hinge. Blue - Radial stop; Green - Radial vein; Red - Pleural wing process; Yellow - Pterale C. Scale bars = 1mm and 0.25mm for the whole thorax and magnified hinge, respectively.

2.4.1 The Gearbox

The earliest mention of a ‘gearbox’ in dipteran insects comes from Pfau (Pfau, 1973). Since this study, many authors have examined the interaction but disagree both in terms of when this interaction occurs, and the result of it. While some have asserted that the interaction occurs during the upstroke and that the main result of this is to assist in the pronation (nose-down pitching) of the wing at the end of the upstroke (e.g. Pfau (1973); Chowdhury and Parr (1981); Pfau (1987) but see Miyan and Ewing (1985b) for a critical review of Pfau’s methods), others asserted that the interaction occurs during the downstroke, and brings about changes in wing kinematics such as varying the stroke amplitude (the angular distance between the wingtip positions at the end of the upstroke and downstroke; Miyan and Ewing (1985a); Wisser (1988); Nalbach (1989); Walker et al. (2012); Deora et al. (2015)).

Of the different theories, those set out by Nalbach (Nalbach, 1989) have remained the most detailed and enduring. Nalbach performed studies on *C. vicina* under tethered flight using stroboscopic illumination to record *RS-PWP* interaction, during which she found four distinct ‘modes’, or ‘gears’, of operation. Gear 3 involved no contact between the *RS* and *PWP*, with the *RS* falling anteriorly of the *PWP* at the end of the downstroke, while gears 2, 1 and 0 each involve some degree of contact. Gears 2 and 1 involved the *RS* fitting into the anterior and posterior grooves of the *PWP*, respectively, while gear 0 involves the *RS* colliding with the posterior shoulder of the *PWP* as illustrated in figure 2.7. (Note that only gears 1-3 are thought to be engaged during the majority of flight, with gear 0 being a much rarer occurrence or artefact of tethering.) The result of any contact was a decrease in stroke amplitude of the associated wing when compared to a gear 3 wingbeat, as well as a delay in the timing of the rapid nose-up pitch movement of the wing (supination) that occurs at the end of the downstroke. This interaction between these two components is therefore thought to be a method through which Diptera are able to elicit rapid changes in wing kinematics.

In a more recent study by Deora and colleagues (Deora et al., 2015), the au-

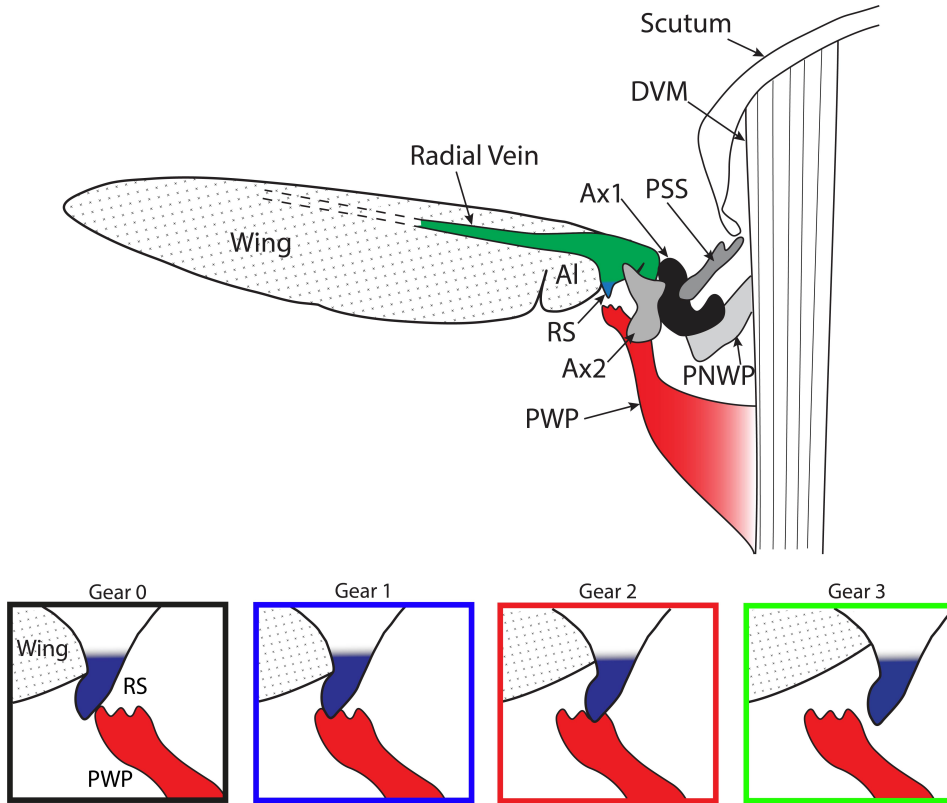


Figure 2.7: Schematic illustrating the anatomy of the gear change mechanism. Top: Simplified cross-sectional diagram of the dipteran wing hinge and wing. Bottom: Illustrations of the *RS* and *PWP* engaging in different gears at the end of the downstroke in *C. vicina*. Abbreviations: *Ax1* - First Axillary Sclerite; *Ax2* - Second Axillary Sclerite; *DVM* - Dorsoventral muscles; *Al* - Alula; *RS* - Radial Stop; *PNWP* - Posterior Notal Wing Process; *PSS* - Parascutal Shelf; *PWP* - Pleural Wing Process. Adapted from Walker et al. (2012).

thors find that gear 0 interactions cause the wings to vibrate, but not necessarily beat. This can occur in *Drosophila* (Bennet-Clark and Ewing, 1968) and *Glossina* courtship song production (Chowdhury and Parr, 1981), or during extreme turns in flight (Nachtigall and Wilson, 1967). Deora et al. use evidence from high speed videos of flesh flies (*Parasarcophaga dux*) to show that this gear precludes wingbeat-like motion. Instead, as the power muscles become active, the *RS* remains locked behind the *PWP* and the wings vibrate instead. In this situation, *RS-PWP* interaction acts almost like the clutch of a car, the engagement of which determines whether or not power from the engine is translated into wheel motion. Any other mode or gear, however, allows the wing to beat.

Deora et al. also used three-dimensional wing trajectory reconstructions and showed that with each change in gear, there was a discrete change in stroke am-

plitude. These results suggest that the gear change mechanism is indeed a method through which wing kinematics can be controlled by a variety of Diptera. The muscular basis that underlies the selection of different gears is complex and not well understood, though this topic will be discussed after a detailed description of the control muscles later in this review (see section 2.6.4).

The biomechanics through which the gear change mechanism brings about these observed changes in wing kinematics have been postulated by the authors who have investigated the interaction, but again different conclusions have been drawn. One school of thought, asserted by Pfau and suggested as a possibility by Wisser (Pfau, 1973, 1987; Wisser, 1987), is that the contact aids wing pitching (the rotation of the wing about its long axis). As the *RS* and *PWP* come into contact towards the end of the downstroke, this contact provides a pivot about which the wing can rotate. Pfau and Wisser suggest that the gear change mechanism is simply a mechanism through which Diptera bring about the observed changes in the timing of wing supination (Nalbach, 1989), and that the other kinematic changes observed across different gears are a result of separate muscular control mechanisms.

Wisser's alternative theory states that *RS-PWP* contact acts as a brake, with the *PWP* inhibiting further movement of the *RS* after the two come into contact, an idea also championed by Miyan & Ewing, Ennos, Nalbach and Deora et al. (Miyan and Ewing, 1985a; Ennos, 1987; Wisser, 1988; Nalbach, 1989). As the *RS* is an extension of the wing's radial vein, this interaction thereby also restricts wing movement. This theory explains many of the differences in wing kinematics that have been recorded across gears (Nalbach (1989); Deora et al. (2015); including Deora et al. 's suggestion of gear 0 acting as a 'clutch')

The requirement for the *PWP* and/or *RS* to absorb energy during the interaction, however, has been a primary counter-argument against this mechanistic theory. Pfau argues that, since both the *RS* and *PWP* are sclerotised components and yet show no visible signs of wear or damage, the two must not come into contact at high speed at the end of the downstroke. The interaction thereby cannot absorb

the energy required to influence wing kinematics such as stroke amplitude (Pfau, 1987). On the other hand, the possibility remains that the *PWP* or *RS* are somewhat elastic structures, and able to absorb this energy without incurring damage. Certain forms of cuticle with elastic properties have been found in other arthropods, particularly in species which store energy during preparation for large and sudden movements, such as jumping or predatory striking (Jensen and Weis-Fogh, 1962; Bennet-Clark and Lucey, 1967; Katz and Gosline, 1992; Gorb, 2004; Vincent and Wegst, 2004; Dudek, 2006; Patek et al., 2007; Burrows et al., 2008; Zack et al., 2009; Burrows and Sutton, 2012). The cuticle at the base of the *PWP* or *RS* may possess elastic properties and allow them to store energy in a similar way. Alternatively, the pleurosternal and tergopleural muscles attaching to the pleural wall (from which the *PWP* originates) may store elastic energy as the *PWP* is bent by *RS* contact (Miyan and Ewing, 1985a; Wisser, 1988). Confirming either of these theories will therefore require further investigations into the structure of both these components, and the details of their interaction.

2.4.2 The Alula

Several features of dipteran flight anatomy distinguish it from that of other insect orders, such as their two winged configuration, the halteres, and the aforementioned gear change mechanism. However, some dipteran insects, for example drone flies (*Eristalis tenax*) are also unique in their ability to change the shape of their wing planform during flight due to a component known as the alula (Walker et al., 2012).

The alula is a proximal part of the wing first identified by Lowne (Lowne, 1890), which varies greatly in size across Diptera (e.g. Colyer and Hammond (1968); Weis-Fogh (1973); Stubbs and Falk (2002); Starý (2008); figure 2.8), occupying up to 10% of the total wing area in some members of the Syrphidae. The alula is also absent from basal members of the order (e.g. the Tipulidae), and other members have secondarily lost it (e.g. the Pipunculidae), suggesting it has some adaptive function in those species, like *E. tenax* and *C. vicina*, that retain it.

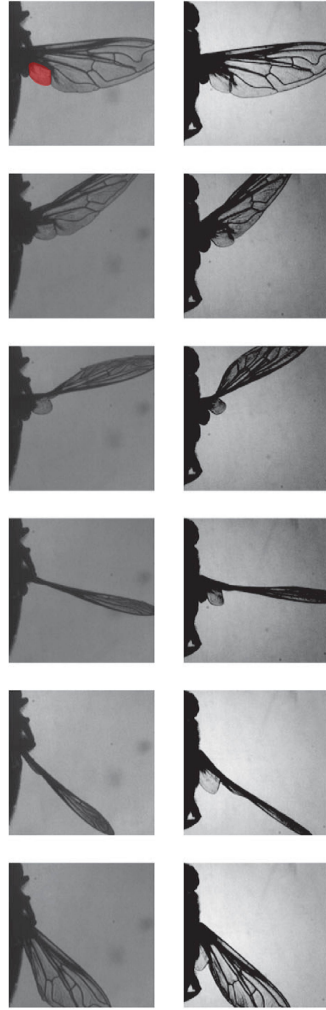


Figure 2.8: Images from videos of two drone flies (*E. tenax*) taken from a dorsal view. The series on the left shows the alula (highlighted in red) operating in its flattened state (except at supination, when it lags behind the rest of the wing), while the right shows the alula operating in its flipped state. Taken from (Walker et al., 2010).

Due to a flexible fold articulating the alula with the rest of the wing, it can occupy either a ‘flat’ state, in which it lies parallel to the rest of the wing, or a ‘flipped’ state in which it stands perpendicular to the wing. The alula occupies this flipped position when the wings are folded over the body at rest, which suggests that this mobility may be an adaptation that subverts a trade off between possessing large wings optimised for flight while at the same time being small enough to fold over the body. However, the alula also changes state during flight, and in a study of these movements in drone flies (*Eristalis tenax*, Linnaeus 1758), Walker and colleagues (Walker et al., 2012) observed significant changes in wingbeat kinematics as the alula changed states during flight. For example, the alula occu-

piated a flat state during high-amplitude wingbeats, while wingbeats in which the alula is flipped exhibited earlier supination at the end of the downstroke compared to alula-flat wingbeats.

The kinematics exhibited during alula-flat wingbeats in Walker et al.’s study were also those that were likely to produce the greatest amount of aerodynamic force due to their large stroke amplitude, for example. Though the flat area of the wing will be larger during alula-flat wingbeats, this increase occurs in an area close to the wing’s root, where the airflow over the wing will be slower during flapping

compared to that at the tip and therefore generating less force (Weis-Fogh, 1973; Walker et al., 2012). Despite this, it is likely that changing the shape of the wing will influence airflow over the wing in some way, but the effects of this are not yet known, though it could have the effect of making the wing more petiolate (Phillips et al., 2017).

Interestingly Walker et al. also noted that these kinematic changes were very similar to those associated with gear changes recorded by Nalbach in *Calliphora* (Nalbach, 1989). The authors therefore suggested that there may be a link between the state of the alula, and the state of *RS-PWP* interaction. Investigating this relationship further may therefore identify the underlying muscular mechanisms controlling the gear change mechanism.

2.4.3 The Gear Change Mechanism: Conclusions

It is clear that Diptera do engage in a form of gear changing during tethered flight, with a discrete set of kinematics associated with each gear and indirect evidence points to their use during free-flight. The likely role of this mechanism is to induce manoeuvres by altering the kinematics of the wings. These changes may be brought about due to *RS-PWP* interaction absorbing kinetic energy from the wing as the two come into contact, or alternatively the interaction may simply provide a pivot about which wing rotation can occur at the end of the downstroke. The unique morphology of this system and its variation across species indicates its likely importance to dipteran flight, and so further study of this system is clearly warranted.

Three chapters in this thesis scrutinise the gear change mechanism and its biomechanics in separate species. Chapter 3 studies the role of the gear change mechanism in the well-studied system of the blow fly, *C. vicina*, while Chapter 4 contrasts the mechanism in this species against that of the drone fly, *E. tenax*. Finally, Chapter 5 assimilates the knowledge from these two prior chapters and studies the movement of the individual components in more detail. From this, I draw conclusions regarding the biomechanics through which the gear change mechanism influences

wing kinematics.

2.5 The Pterale C

During the study of the gear change mechanism, many authors have also noticed the radial stop interacting with another poorly understood component of the wing hinge known as the Pterale C (Ritter, 1911). This club-like cuticular projection has a bulbous head which quickly tapers down to a much thinner base (figure 2.9), but the significance of this morphology is unknown as it has received very little attention in the literature. The few studies that have mentioned the Pterale C (*PtC*) have observed that its large head makes contact with a groove on the anterior side of the *RS* during the end of the downstroke, which has led to a great deal of speculation regarding the function of this contact.

The only study that has been devoted entirely to the *PtC* comes from Miyan & Ewing (Miyan and Ewing, 1984), who found that, in *Drosophila virilis*, the head of the *PtC* is lined with numerous cuticular evaginations. These, along with its club-like anatomy, translating large distal movements into small proximal movements, are typical of sensory organs (Keil, 1997). However, it should be noted that these evaginations have never been found to have any sensory capacity, and appear to simply be outgrowths of the cuticle. Despite this, Miyan & Ewing did find sensory nerve activity from the base of the *PtC* as it was moved. From these findings, and the anatomy of the *PtC*, Miyan & Ewing conclude that it is a wingbeat-synchronous proprioceptor that is stimulated by movement of the wing towards the end of the downstroke. This conclusion has been supported by several studies which have mentioned the *PtC* (Miyan and Ewing, 1985a; Nalbach, 1989; Fayyazuddin and Dickinson, 1996).

Nalbach agreed with Miyan & Ewing regarding the sensory capacity of the *PtC*, but also suggested that it could play an alternative, mechanical role of a shock absorber (Nalbach, 1989). A mechanical role for the *PtC* had already been mentioned by other authors (Chowdhury and Parr, 1981; Wisser, 1988), who have suggested

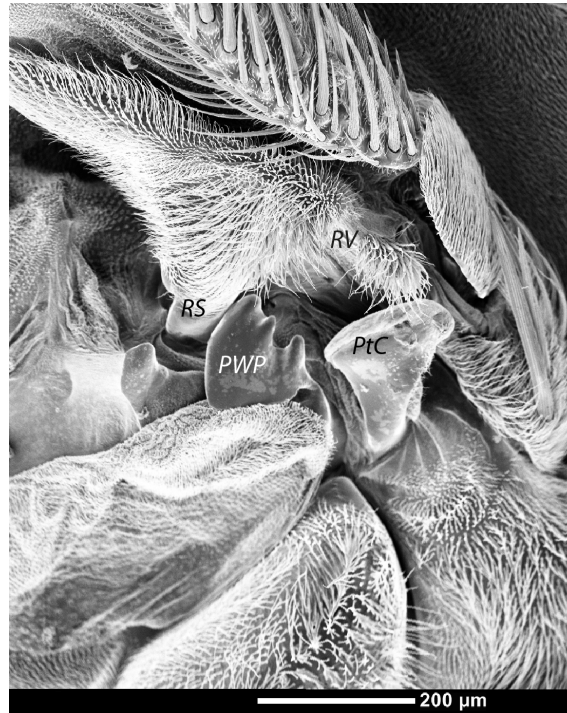


Figure 2.9: An SEM image of the right wing hinge of *C. vicina* showing the detail of the wing hinge (see appendix A.5 for details). Abbreviations: *PtC* - Pterale C; *PWP* - Pleural Wing Process; *RS* - Radial Stop; *RV* - Radial Vein. See appendix A.5 for methodological details.

that the structure's role could lie in aiding in courtship song production, or wing rotation at the end of the downstroke. Nalbach asserted that, because the *RS* and *PWP* fail to make contact during gear 3 wingbeats (section 2.4), some alternative mechanism of stroke amplitude control might be required. Coincidentally, the amount of *RS-PtC* contact was also most extensive during gear 3 wingbeats, leading to the conclusion that the *PtC* may absorb some of the kinetic energy of the wing during this contact to either slow it down or limit its movement as it approaches wing reversal. There is also evidence that the *PtC* can be moved independently of the wing hinge by the activity of the muscles attaching to the basalar sclerite (Miyan and Ewing, 1985a; Nalbach, 1989), meaning these muscles could influence the timing of *RS-PtC* contact. In the scorpion fly *Panorpa*, which lacks a well defined *PtC*, Ennos describes a dorsal sclerotisation of the basalar sclerite which may be homologous to the *PtC* and is thought to act as a downstroke-limiter in a similar way to the gear change mechanism (Ennos, 1987). Since the *RS-PWP* interaction is thought to fill this downstroke limitation role in *Calliphora* the *PtC*

may be emancipated from this mechanical role and fill a sensory function instead.

It is important to note that neither the sensory nor mechanical functions suggested for the *PtC* are exclusive of each other, so it remains a possibility that the *PtC* is capable of performing both. Given the prominence of the *PtC* within the wing hinge, and its possible proprioceptive or shock-absorbing role, this neglected component of the flight motor deserves more attention. In Chapter 6, I make use of high-speed macrovideography and three-dimensional wing kinematic tracking to study the movement of the *PtC* and its interaction with the *RS* in order to determine the validity of these competing theories.

2.6 The Flight Control Musculature

The power muscles are capable of producing a basic, symmetrical wingbeat, but it is the role of the control muscles to make alterations to this wingbeat. These control muscles can be grouped into two types: the direct and indirect muscles. The direct muscles (sometimes referred to as ‘steering muscles’) attach to some of the sclerites in and around the wing hinge, while the indirect control muscles attach elsewhere in the body, meaning they cannot directly affect the configuration of the wing hinge. Instead, activity of these muscles changes wing kinematics by altering the properties of the whole thorax, such as its stiffness.

The direct muscles attach to three of the four sclerites that make up the wing hinge. The first and third axillary sclerites (*Ax1* and *Ax3*, respectively), and the posterior notal wing process (*PNWP*) all have between two and four muscles attaching to them (figure 2.2), while the second axillary sclerite (*Ax2*) has none. Another, the basalar sclerite, is found just anterior to the wing hinge and has three muscles that attach to it.

These muscles can be further divided into ‘tonic’ muscles, which are active on almost every wingbeat, and ‘phasic’ muscles which are active much more infrequently (Lindsay et al., 2017). Each of *Ax1*, *Ax3*, the *PNWP* and the basalar sclerite appear to have at least one of each muscle type attaching to them, and Lindsay et al. (Lind-

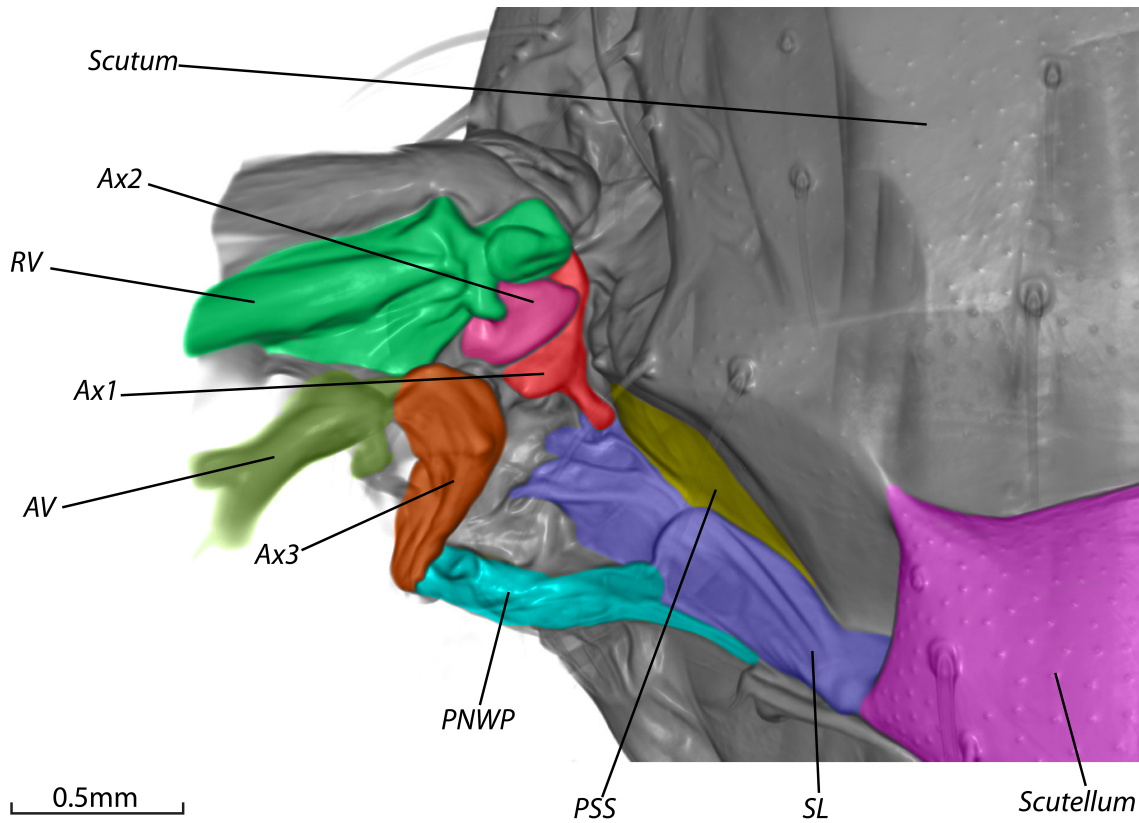


Figure 2.10: A μ CT scan image of a *C. vicina* from a dorsal view, highlighting various components of importance in this review. Abbreviations: *AV* - Wing's anal veins; *Ax1* - First axillary sclerite; *Ax2* - Second axillary sclerite; *Ax3* - Third axillary sclerite; *PNWP* - Posterior notal wing process; *PSS* - Parascutal shelf; *RV* - Wing's radial vein; *SL* - Scutellar lever. Scale bar = 0.5mm.

say et al., 2017), having used real-time calcium imaging to visualise muscle activity in *Drosophila* during flight, suggest that the two types are specialised in their size and activity patterns to perform different roles. Where the tonic muscles are small, active during almost every wingbeat and vary in the timing of this activity during each wingbeat, phasic muscles are larger, active more rarely and show less variation in activation timing compared to tonic muscles (e.g. Heide (1983); Fayyazuddin and Dickinson (1999); Balint and Dickinson (2001)). This leads Lindsay et al. to assert that tonic and phasic muscles are adapted to exert fine and coarse control over sclerite movement, respectively. These various muscles with different properties are all able to act in concert to ensure that the movement of the sclerites are under fine control. Combined with the activity of the indirect control muscles, these muscles exercise precise control over the wing kinematics.

Extensive research has been performed to identify the activity patterns and ef-

fects of these muscles on the wing kinematics, which is summarised in table 2.1. However, there are still aspects of the muscular control complex which are not well understood, particularly the biomechanics through which these muscles bring about their observed effects on wing motion.

2.6.1 Direct Control Muscles

The First Axillary Sclerite

The first axillary sclerite (*Ax1*) articulates with three structures of major importance in the flight motor: the scutellar lever, the parascutal shelf, and the second axillary sclerite, *Ax2*. As well as this, *Ax1* connects to the radial vein of the wing (figures 2.10 & 2.11, Chapman (2013)). Movements of *Ax1* are therefore responsible for driving the basic wingbeat, and so any alteration of this sclerite's motion will have a significant effect. *Ax1* has two muscles attaching directly to it (figure 2.2A). The first muscle (*I1*) originates from the dorsal episternum, and attaches to the inner arm of *Ax1*, while the second (*I2*) originates on the pleural apophysis (a ridge found on the interior side of the pleurum) and again attaches to the inner arm of *Ax1* (see Wisser and Nachtigall (1984) for a detailed description).

Ritter (Ritter, 1911), in one of the first detailed anatomical descriptions of *Caliphora*, identified only *I1*, and through physical manipulation of the muscle claimed that its role was to raise the wing and draw it backward. Later studies using electrophysiology found that *I1* was active only during ipsilateral turns (reviewed in Heide (1983); Heide (1975); Egelhaaf (1989); Heide and Götz (1996)), implying that its activity could alter wing kinematics sufficiently to induce a torque. More detailed investigations found that it achieves this by reducing the anterior movement of the wing at the end of the downstroke (Balint and Dickinson, 2001), lowering the stroke plane (Lindsay et al., 2017), and changing the downstroke deviation angle (defined in these studies as the variation in downstroke trajectory from the antero-posterior body axis; Balint and Dickinson (2004)). Together, these kinematic changes imply that *I1* is involved in roll manoeuvres during flight.

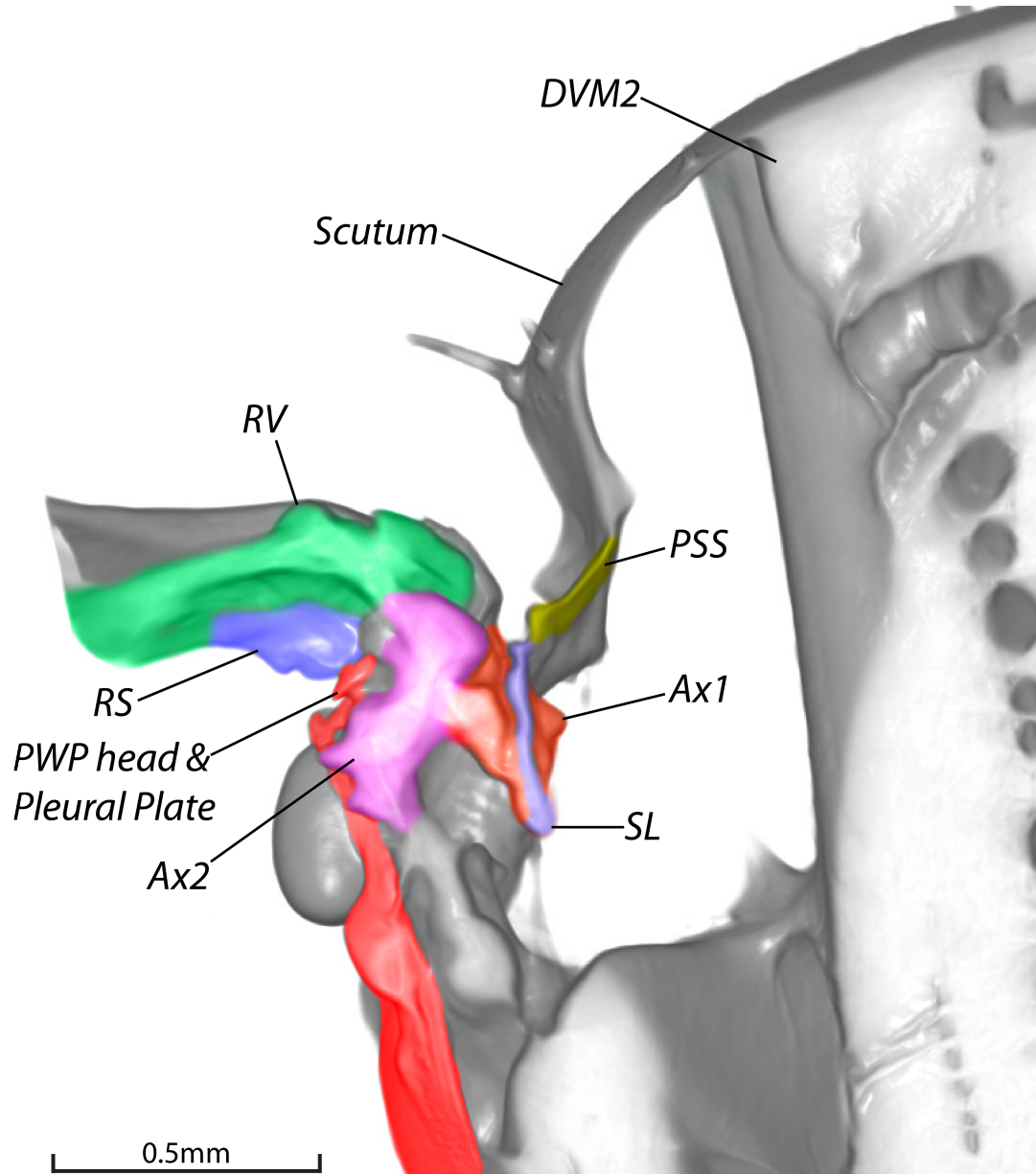


Figure 2.11: Cross section of a μ CT scan image of the left wing hinge of a *C. vicina* thorax, with various components of the hinge highlighted. Abbreviations: *Ax1* - First axillary sclerite; *Ax2* - Second axillary sclerite; *DVM2* - Second dorsoventral muscle; *PSS* - Parascutal shelf; *PWP* - Pleural wing process; *RS* - Radial stop; *RV* - Radial vein; *SL* - Scutellar lever. Scale bar = 0.5mm.

Table 2.1: A summary of the different control muscles, whether they are phasic or tonic according to Lindsay et al. (Lindsay et al., 2017), the sclerites to which they attach, and the effects of their activity on wing movement as found by previous authors. References: [1] - Ritter (1911); [2] - Nachtigall and Wilson (1967); [3] - Heide (1975); [4] - Kutsch and Hug (1981); [5] - Heide (1983); [6] - Wisser and Nachtigall (1984); [7] - Miyan and Ewing (1985a); [8] - Egelhaaf (1989); [9] - Nalbach (1989); [10] - Dickinson et al. (1993); [11] - Heide and Götz (1996); [12] - Tu and Dickinson (1996); [13] - Dickinson and Tu (1997); [14] - Balint and Dickinson (2001); [15] - Balint and Dickinson (2004); [16] - Walker et al. (2017). * *III2* was not studied by Lindsay et al. as the muscle is not present in *Drosophila*.

Control Muscle Group	Muscle	Origination Site	Muscle Type	Effects of Muscular Activity
Basalar Sclerite	<i>b1</i>	Anterior dorsal episternum (near notopleural cleft)	Tonic	Moves the wing forward, moves the wing into a flight-ready position [1, 6]; increases activity spike frequency during contralateral turns [5], decreases spike frequency during ipsilateral turns [3, 5]; active during high amplitude wingbeats [11, 12, 16]; increases anterior wingtip movement in downstroke [9, 17]; raises the downstroke deviation angle [12, 14, 15]; may control wing pitch angle [10].
	<i>b2</i>	Ventral parts of anterior dorsal episternum	Phasic	Moves the wing forward [1, 6]; fires while ipsilateral wing is beating, quiescent while not beating [2]; increases activity during turns to the contralateral side [5], decreases activity during turns to ipsilateral side [3]; active during high amplitude wingbeats [11, 12, 16]; increases anterior wingtip movement in downstroke [9, 17]; raises the downstroke deviation angle [12, 14, 15]; may control wing pitch angle [10].
	<i>b3</i>	Posterior dorsal episternum (posterior of wing hinge)	Tonic	Associated with low amplitude wingbeats [16]; increases posterior movement of the wingtip in upstroke [17].
First Axillary Sclerite (<i>Ax1</i>)	<i>I1</i>	Dorsal Episternum	Phasic	Active during ipsilateral turns [3, 5, 8], decreases wingbeat amplitude [11, 14, 16, 17]; decreases anterior movement of the wingtip during the downstroke [15] lowers stroke plane [17].
	<i>I2</i>	Pleural apophysis	Tonic	Lowers stroke plane and decreases wingbeat amplitude [17].
Third Axillary Sclerite (<i>Ax3</i>)	<i>III1</i>	Dorsal episternum	Phasic	Raises the wing and move it backward [1]; active during contralateral turns [3, 5, 8], though see [11]; associated with decreased stroke amplitude [16]; raises stroke plane and active at the end of flight as the wing is folded over the back [17].
	<i>III2</i>	Pleural apophysis	Not studied*	Increase stroke amplitude [14]; increases anterior wingtip movement during downstroke [15]; raises stroke plane [17].
	<i>III3</i>	Dorsal episternum	Tonic	Depresses anal portion of the wing [1]; increases stroke amplitude [14]; increases anterior wingtip movement during downstroke [15]; raises stroke plane [17].
	<i>III4</i>	Anterior dorsal episternum	Phasic	Increases stroke amplitude [14]; increases anterior wingtip movement during downstroke [15]; raises stroke plane [17].
Posterior Notal Wing Process (PNWP)	<i>hq1</i> <i>hq2</i>	Pleural apophysis	Phasic Phasic	Brings the wing to a resting position [1]; active while ipsilateral wing is at rest, but contralateral wing is beating [2], reviewed in [13]; involved in wing pitch angle control [17]. Involved in wing pitch angle control [17].
	<i>hq3</i>	apophysis (near pleural ridge)	Phasic	Depresses anal portion of the wing [1]; active during ipsilateral turns [3]; involved in wing pitch angle control [17].
	<i>hq4</i>	Dorsal Epimerum	Tonic	Involved in wing pitch angle control [17].
Pleurosternal	<i>ps1</i>	Meso-sternal furca to pleural apophysis	Not studied.	Increases wingbeat frequency [2, 4, 5, 6].
	<i>ps2</i>	Mesothoracic furca to pleural apophysis		Unknown.
Tergopleural	<i>tp1</i>	Dorsal episternum (anterior of <i>PWP</i>) to central scutum	Not studied.	Individual effects unknown, but could play a role in <i>RS-PWP</i> interaction [7]; increases overall tension within the thorax [6].
	<i>tp2</i>	Antero-ventrally of the pleural ridge to central scutum		

The biomechanics of *I1* were studied by Walker et al. (Walker et al., 2014), who found that the tendon attaching the muscle to *Ax1* buckled during the upstroke. Buckling is an unusual behaviour for a muscle-tendon unit as it limits the period over which it can do work. This feature can, however, be beneficial, as *I1* cannot exert any tensile force when buckled, and can therefore only affect wing kinematics once it has assumed a taut state again. This is particularly useful if the muscle's twitch duration is not fast enough to produce wingbeat synchronous contractions (a feature that has been observed in the control muscles during tethered flight: Tu and Dickinson (1994)). Furthermore, this buckling permits a large range of motion of *Ax1* while still maintaining a small strain amplitude of the muscle itself. The anatomy of *I1* thereby enables it to function usefully within the flight motor where a large amplitude of motion is required despite the muscle only being capable of small strain changes, and this muscle therefore only does work during the phase of the wingbeat during which it is subject to tensile loading.

I2 has been comparatively poorly studied. Only a very recent study by Lindsay et al. has revealed that it influences the wingbeat in a similar way to *I1*, by decreasing stroke amplitude and lowering the stroke plane (Lindsay et al., 2017), meaning that it is also involved in roll manoeuvres. Like *I1*, *I2* also possesses a large tendon (figure 2.2; Williams and Williams (1943); Nachtigall and Wilson (1967); Wisser and Nachtigall (1984); Miyan and Ewing (1985a)), so it is likely that this muscle also exhibits the same buckling pattern of motion. A more detailed understanding of these muscles can be gleaned through the use of novel techniques such as time-resolved synchrotron X-ray microtomography (Walker et al., 2014), which will be utilised later in this thesis to determine the biomechanics of these muscles and their roles within the flight motor (Chapter 7).

The Third Axillary Sclerite

The third axillary sclerite (*Ax3*) is found more distally in the hinge than *Ax1* (figure 2.10), but its effects on wing movement are still pronounced. *Ax3* articulates within

the wing hinge proximally with the *PNWP*, and distally with the anal veins of the wing (Chapman, 2013). Four muscles attach to *Ax3*, and all connect to the sclerite via a common tendon, but the muscles themselves originate from different parts of the thorax. The first (*III1*) attaches to the dorsal episternum, the second (*III2*) attaches to the pleural apophysis, the fourth (*III4*) originates on the anterior dorsal episternum, and the third (*III3*) attaches between the second and fourth muscles (see Wissner and Nachtigall (1984) for a detailed description; figures 2.10 & 2.2).

Electrophysiological studies (reviewed in Heide (1983)) have shown that *III1* is associated with contralateral turns in *Calliphora* and *Musca* (Heide (1983); Egelhaaf (1989); though not in *Drosophila*, see Heide and Götz (1996)). Though these authors could not determine the exact kinematics underlying this, Lindsay et al. found that the *III1* activity may raise the stroke plane of the wingbeat, which could be responsible for the contralateral turning torque (Lindsay et al., 2017). Contrary to this, however, Walker et al. studied *III1* and found a lower mean strain in the muscle while the wing was moving with a low stroke amplitude (compared to the strain associated with a high amplitude wingbeat), which would likely result in an ipsilateral turn (Walker et al., 2014). These differences in conclusions across studies could, however, be confounded by other nearby muscle activity, whether through electrophysiological crosstalk or mechanical coupling.

Despite their small size and tightly grouped morphology, the second, third and fourth muscles of *Ax3* have also been studied in detail using similar techniques. Balint & Dickinson (Balint and Dickinson, 2001) found that *III2-4* function synergistically with the muscles of the basalar sclerite, in that for any given activity of the basalar muscles (found to increase wingbeat amplitude in this study) the forward amplitude of each stroke was further increased when *III2-4* were active. Balint & Dickinson suggest that *III2-4* alter wing kinematics by varying the state of the gear change mechanism, an idea supported by Walker et al. 's (Walker et al., 2012) study on *E. tenax* (section 2.4). On the other hand, Lindsay et al. 's recent study on muscular activity in *Drosophila* found that, like *III1*, the role of *III2-4* may be to

raise the stroke plane of the wingbeat (Lindsay et al., 2017). Lindsay and colleagues were also able to determine that, though the function of each *Ax3* muscle is similar, *III1* and *III4* were classed as tonic muscles as they were active only rarely (generally becoming active during large kinematic changes), whereas *III3* was classed as phasic, as it became active on every wingbeat (*III2* was not documented in this study as it could not be located in the genus studied, *Drosophila*). These muscles are therefore adapted to perform different roles through their activity timings and their anatomy, both of which affect their ability to do work.

The roles of the *Ax3* muscles are fairly well understood, but given the contradictory results regarding *III1* function by different authors, further study of this muscle is clearly warranted. The biomechanics of this group are particularly interesting due to the correlations identified between this muscle complex and the gear change mechanism, possibly enabling these muscles to affect wing kinematics using the mechanical interaction of the radial stop and pleural wing process (see section 2.4).

The Posterior Notal Wing Process

Like *Ax1* and *Ax3*, the posterior notal wing process (*PNWP*) is located within the wing hinge (figure 2.10). The *PNWP* is a structure of the pleural wall (figure 2.10), but in dipterans it also includes the fourth axillary sclerite, which is fused to it. Four muscles attach to the *PNWP* (Wisser and Nachtigall, 1984; Dickinson and Tu, 1997): The first (*hg1*) is attached via a long tendon to the pleural apophysis (a strengthening ridge found on the interior of the pleurum), from which it runs dorso-laterally to the outer wall of the *PNWP*. The second, third and fourth muscles (*hg2-4*) all attach via a common tendon which runs dorso-medially from their origins. The origin of *hg2* can be found laterally on the pleural apophysis, *hg3* attaches on the pleural apophysis where it joins the pleural ridge, and that of *hg4* attaches to the *PNWP* from the dorsal epimerum (figures 2.10 & 2.2; Wisser and Nachtigall (1984)).

The muscles of the *PNWP* have been relatively poorly studied in comparison to those of *Ax1* and *Ax3*, most likely due to their relatively small size and inaccessibility in the thorax. Studies by Heide (Heide, 1975) showed that *hg3* was normally inactive during straight flight in both *Musca* and *Calliphora*, but became excited during ipsilateral turns. The precise kinematics underlying these manoeuvres were not recorded, but Lindsay et al.’s recent study on *Drosophila* found that muscular activity influenced the wing pitch angle (Lindsay et al., 2017).

During more extreme wing movements during tethered flight, Nachtigall & Wilson (Nachtigall and Wilson, 1967) observed that *hg1* was active when the ipsilateral wing was not beating, despite the contralateral one undergoing movement (reviewed briefly in (Dickinson and Tu, 1997)). Shirangi et al. also show that *hg1* is sexually dimorphic in *Drosophila* and its activity is a requirement for courtship song production (Shirangi et al., 2013). The results of these studies imply that some muscles of the *PNWP* are responsible for holding the wing in a resting position, and while it remains active it will prevent wing movement. In this case, power muscle activity causes a vibration rather than a wingbeat. Furthermore, this suggests that perhaps the *PNWP* is part of the ‘clutch’ mechanism suggested by Deora et al. (Deora et al., 2015), who assert that the activity of certain muscles permit the wings to move in a wingbeat fashion when the power muscles become active, while others restrict the wings to simply vibrate instead (as occurs during *Glossina* courtship song Chowdhury and Parr (1981)). Deora et al. liken this situation to the clutch of a car, the engagement of which is required for power from the engine to be translated into rotation of the wheels.

The Basalar Sclerite

Unlike the rest of the sclerites discussed here, the basalar sclerite is not found within the wing hinge itself, but is instead found just anterior to the wing hinge. The triangular head of the sclerite is visible externally at the junction between the notopleural and episternal clefts (figures 2.4 & 2.12; Wisser and Nachtigall (1984); Tu and Dick-

inson (1994)). Attached to the triangular head is a rod-like structure that projects medially into the body, from which three control muscles originate. The first (*b1*) attaches anteriorly to the anterior dorsal episternum near the notopleural cleft; the second (*b2*) attaches antero-laterally to the ventral parts of the anterior dorsal episternum; and the third (*b3*) attaches posteriorly to the posterior dorsal episternum behind the wing hinge (figures 2.2 & 2.12; Tu and Dickinson (1994); for a detailed description see Wisser and Nachtigall (1984)). The orientation and attachment of these muscles mean that *b1* and *b2* have the effect of rotating the basalar sclerite anteriorly, while *b3* has an antagonistic, posteriorly rotating effect.

A second major component is known as the ‘basalar 2’ (*Ba2*), which appears as a postero-dorsal outgrowth from the main body of the basalar sclerite (figure 2.12). A critical feature of the *Ba2* is the ligament that connects it to the base of the wing’s radial vein. This ligament provides a direct connection between the wing and the sclerite. This both ensures that the sclerite undergoes movement in time with the wing, but also means that the wing can be directly controlled by movements of the basalar sclerite, indicating the importance of this component to dipteran flight control.

The first investigation of the anatomy and function of *b1-3* comes from Ritter (Ritter, 1911), but it was not until later studies that the role of the muscles were understood in great detail. Work on *Muscina*, for example, found that *b1* fired on every wingbeat, while *b2* was not as consistently active, and increases or decreases in activity were associated with contralateral or ipsilateral turns respectively. The two muscles also show phase dependence, in that the timing of muscle activation can be altered, which results in changes to wing kinematics (Heide, 1975, 1983; Egelhaaf, 1989; Lehmann and Götz, 1996; Dickinson et al., 1993; Heide and Götz, 1996). An earlier activation of either muscle, for example, is associated with an earlier supination of the ipsilateral wing.

Tu & Dickinson (Tu and Dickinson, 1994) continued the investigation, finding that the power output of the muscle was negative when undergoing induced os-

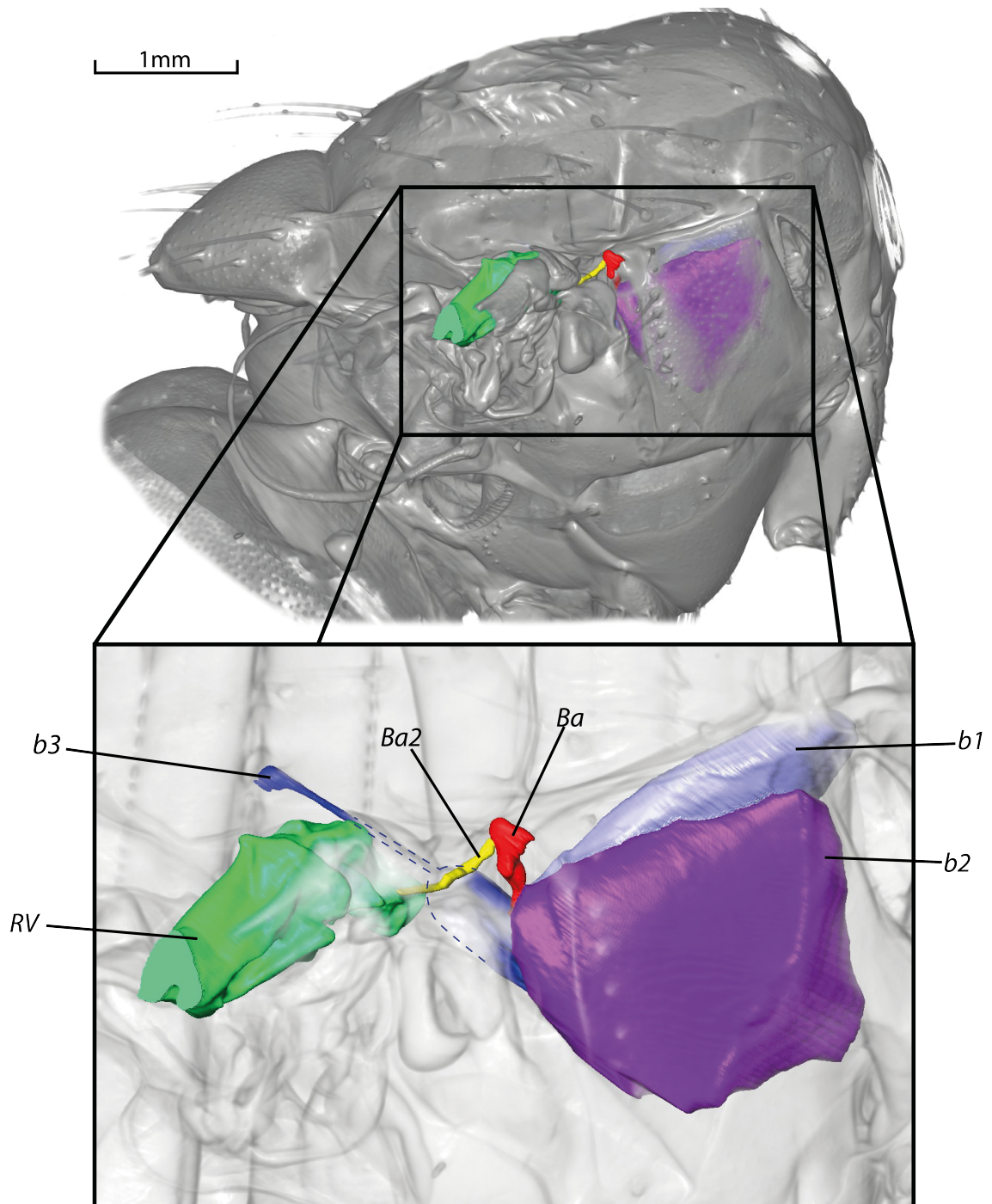


Figure 2.12: A μ CT scan image of the basalar sclerite-muscle complex of *C. vicina*, illustrating the position of the sclerite and the attachment sites of the three basalar muscles. The ligament that connects the basalar sclerite via the basalar 2 is also shown. Abbreviations: *b1-3* - Muscles 1-3 of the basalar sclerite; *Ba* - The basalar sclerite, with its triangular head and medially projecting rod-like body; *Ba2* - The basalar 2 component and ligament connecting to the radial vein; *RV* - Radial vein. Scale bar = 1mm.

cillations at wingbeat frequency, mean that it was acting to resist movement by absorbing energy (i.e. it performed negative work). Physiological studies reveal that muscles are able to perform negative work more effectively and efficiently than they are positive work (Fenn, 1924; Abbott et al., 1951, 1952; Bigland-Ritchie and Woods, 1976; Lindstedt et al., 2001; Elmer and LaStayo, 2014). The physiology underlying this is unclear, though it may stem from the fact that actin-myosin bonds within the muscle filaments can detach and cycle without the need for metabolic energy input (Menard et al., 1991; Pinniger et al., 2006), from the elastic properties of the individual muscle units (sarcomeres) which resist lengthening, or from the interactions between filaments within the sarcomere as they unwind from each other, otherwise known as the ‘winding filament’ hypothesis (reviewed in Nishikawa et al. (2012); Nishikawa (2016); Hessel and Nishikawa (2017)). Though Tu & Dickinson found that the contraction cycle of *b1* is too slow relative to wingbeat frequency to permit positive work, the benefits of performing negative work mean that this is likely an adaptation of this muscle and not a restriction imposed by the rapid oscillations of the system.

Restricting basalar sclerite movement through the negative work of *b1* (and possibly the other basalar muscles) appears to regulate wing movement by changing the anterior offset of the wingtip trajectory at the end of the downstroke in *Calliphora* (Tu and Dickinson, 1996). This finding is further corroborated by a more recent study by Lindsay et al. in *Drosophila* which used real-time calcium imaging to visualise muscle activity in flight (Lindsay et al., 2017). *b2* activity was found to have had a similar effect, though also increasing the amount of stroke deviation (defined as wingtip deviation from the stroke plane: the plane connecting the positions of the wingtips at the end of the upstroke and downstroke) of the ipsilateral wing.

Modern techniques continue to build on this knowledge, with Lindsay et al. finding that *b3* activity was correlated with a greater posterior offset of the wingtip at the end of the upstroke. Walker et al. (Walker et al., 2014) used different methods to visualise the muscle’s movement, and found that the muscle exhibited less strain

during low amplitude wingbeats. This study also revealed an unexpected behaviour of the basalar sclerite itself, in that the internal segment of the sclerite appears to follow a path aligned with *b1* during a low amplitude wingbeat, and one aligned with *b3* during a high amplitude wingbeat. This movement of the sclerite increases the amount of negative work *b1* can perform and thereby could allow *b1* to absorb a greater amount of kinetic energy from the wing (which was ultimately produced by the power muscles) to produce low amplitude wingbeats.

2.6.2 Indirect Control Muscles

The Pleurosternal and Tergopleural Muscles

Unlike all the other muscles mentioned so far in this review, the pleurosternal and tergopleural muscles do not attach into any sclerites which are part of or nearby the wing hinge. Instead, they attach internally to either side of the thorax, and as they contract they change the properties of the thorax, for example by increasing its stiffness. The first pleurosternal muscle (*ps1*) attaches between a process that projects dorso-anteriorly from the episternum (the meso-sternal furca) and the edge of the pleural apophysis. The second (*ps2*) attaches to a similar projection of the internal part of the episternum (the mesothoracic furca), then projects dorso-caudally as it transitions into a tendon. This then inserts onto the posterior passage of the pleural apophysis into the pleurum (for a detailed description, see (Wisser and Nachtigall, 1984); figure 2.2).

The tergopleural muscle, on the other hand, has two morphological subunits. The first originates in a vault on the dorsal episternum anterior to the pleural wing process, while the second begins ventrally anterior to the pleural ridge (a thickened section of the pleurum) and surrounds the first subunit caudally as a small muscle band. Both of these subunits unite to form a tendon that ends on a sclerotised ledge of the central part of the scutum (Wisser and Nachtigall, 1984).

When the pleurosternal muscles are stimulated, wingbeat frequencies increase nearly linearly with spike frequency in the muscles (Nachtigall and Wilson (1967);

Kutsch and Hug (1981); Wisser and Nachtigall (1984); reviewed in Heide (1983)). The muscles are thought to increase the stiffness of the thorax when contracted, which increases the resonant frequency of the system. This causes more rapid oscillations of the thorax deformation driven by the power muscles and greater wingbeat frequencies as a result (Dudley, 2000). This is likely to be a symmetrical change, and it is not yet known whether the muscles on either side of the body are capable of inducing asymmetries in wing kinematics.

The tergopleural muscle (*tp*) has received comparably little attention. Wisser & Nachtigall (Wisser and Nachtigall, 1984) claim that it acts as both a tensioning muscle (like the pleurosternals) and a steering muscle, while Miyan & Ewing (Miyan and Ewing, 1985b) propose that the muscle may act to rotate *Ax1* by causing small relative movements between the scutum and the pleural wing process (figures 2.12 & 2.7). They also suggest that the *tp* is involved in determining the selection of ‘gears’ in the gear change mechanism (part 2.4 of this review; Nalbach (1989); Walker et al. (2012)). The actual role of the *tp* will therefore require more investigation to determine, but the use of time-resolved synchrotron X-ray microtomography should enable future studies to examine *tp* movement during flight and identify a causal relationship between this and changes in wing kinematics.

2.6.3 Flight Initiation

Initiating flight in the Diptera requires some mechanism to begin the self-sustaining contraction cycle of the power muscles (see section 2.3). Much attention has therefore been given to muscles which are capable of lengthening the stretch-activated DVMs and DLM. As the DVMs and DLM act antagonistically it is possible that the activation of either one of these groups could initiate flight. Exactly this has been observed in *Drosophila*, in which escape responses from looming object stimuli triggers activity in the giant fibre neuron pathway (Koto et al., 1981), and this in turn leads to activity in the DLM (Tanouye and Wyman, 1980; King and Wyman, 1980; Augustin et al., 2011).

The giant fibre neuron pathway has also been shown to activate another muscle; the tergotrochanter (*tt*; Power (1948); Mulloney (1969); Levine and Tracey (1973); Tanouye and Wyman (1980); King and Wyman (1980); Koto et al. (1981); Thomas and Wyman (1982); Miyan and Ewing (1985a); Trimarchi and Schneiderman (1993); Augustin et al. (2011), but see (Bacon and Strausfeld, 1986)). The *tt* attaches to a central area of the scutum, the mesoscutum, very close to that of *DVM2*. It then becomes a sclerotised tendon which attaches near the origination site of the muscles of the legs on the trochanter of the mesothoracic legs, the middle pair of legs on the thorax (figure 2.2; see Wisser and Nachtigall, 1984, for a detailed description (Wisser and Nachtigall, 1984)). As the *tt* contracts, it causes the legs to extend, initiating a jump that often precedes flight (Nachtigall and Wilson, 1967; Mulloney, 1969; Miyan and Ewing, 1985a; Holmqvist, 1994) and also deforms the thorax slightly in a similar way to DVM activity. By this merit, it also stretches the DLM (Nachtigall and Wilson, 1967; Miyan and Ewing, 1985a; Augustin et al., 2011). In stretching the DLM, this causes them to contract and begins the self-sustaining wingbeat cycle (section 2.3). This feature has lead some authors to term the *tt* ‘the starter muscle’ (Nachtigall and Wilson, 1967; Mulloney, 1969).

That the *tt* is the sole muscle responsible for initiating flight is not a universal conclusion. Several authors have observed that flight is still possible even when the muscle has been severed (Nachtigall and Wilson, 1967; Bennet-Clark and Ewing, 1968; Heide, 1968; Schouest et al., 1986) or in species where it is completely absent, such as *Glossina morsitans* (King, 1983) or long-legged nematocera such as *Tipula* (Smart, 1959). Furthermore, the *tt* is not always active before or during the beginning of flight in *Musca* (Holmqvist, 1994), and several studies have observed activity in the DVMs or DLM instead of the *tt* in *Drosophila* (Trimarchi and Schneiderman, 1995; Augustin et al., 2011).

While the evidence available suggests that the *tt* is unlikely to be the sole ‘starter’ muscle in the flight motor, it is still very well suited to stimulate both a jump to provide ground clearance and the required power muscle activity to begin and maintain

flight. Given that the giant neuron pathway is seen to stimulate the *tt* in response to some forms of looming stimuli (Holmqvist, 1994), this muscle could be involved in eliciting certain evasive manoeuvres, while different muscles are responsible for others. Other starter muscle candidates have been put forward, such as the pleurosternal muscles (Schouest et al., 1986), but further study of these possibilities is still warranted.

There are further complexities in the flight motor, however, such that it is not sufficient for the power muscles to become active to initiate flight. Nachtigall & Wilson (Nachtigall and Wilson, 1967) and Tu & Dickinson (Tu and Dickinson, 1994), for example, studied the muscles of the basalar sclerite before flight, and found that if both *b1* and *b2* become quiescent while the indirect flight muscles are still active the wings vibrate, but do not flap. This insinuates that activity of the basalar muscles is essential for moving the wing and wing hinge into a position in which flight is possible. Similarly, Tanouye & King (Tanouye and King, 1983) also found that activity in *b2* (*pa3* by their nomenclature) was associated with flight initiation in *Drosophila*. Like the muscles of the *PNWP*, the muscles of the basalar sclerite could therefore be part of the clutch mechanism discussed by Deora et al. (Deora et al., 2015), in that activity of these muscles create a wing hinge configuration that facilitates the translation of power muscle activity into a wingbeat.

The wealth of work in this area shows that flight initiation in the dipteran flight motor appears to be comprised of two stages: an engagement of a clutch-like mechanism that brings the wing hinge into a configuration that is amenable to wing motion; and the stimulation of activity in the power muscles. The first stage is accomplished through the activity of the muscles of the basalar sclerite and *PNWP*, while the second stage is achieved through the action of the *tt* (or other muscles) stretch-activating the power muscles, or through the direct stimulation of the power muscles themselves. This mechanistic explanation is, however, simplified, and further research is needed to reveal the complex sclerite interactions involved in this behaviour.

2.6.4 Muscular Control of Gear Changes

The muscular mechanisms underlying the selection of different gears is quite poorly understood. However, several theories have been put forward by those studying the mechanism, leading to two main schools of thought: the first being that gears are selected by changing the position of the *PWP*, and the other being that changes to the wing trajectory causes *RS-PWP* interaction.

Pfau (Pfau, 1973), for example, found that the tergopleural muscle attaches to the internal rod of the *PWP* from the central part of the scutum, the mesoscutum (Wisser and Nachtigall, 1984). He claims that this muscle is capable of moving the *PWP* forwards and inwards, thereby changing its orientation relative to the *RS*; a theory also shared by Wisser (Wisser, 1988) and Miyan & Ewing (Miyan and Ewing, 1985a, 1988).

Nalbach (Nalbach, 1989), on the other hand asserted that changes in wingbeat paths were responsible for gear selection. She focused on the basalar sclerite, observing that changes in activity of *b1* and *b2* resulted in a decreased stroke amplitude. These observations suggest that under some flight conditions, the muscles act to move the wings in a path such that the *RS-PWP* contact during the downstroke, while during other wingbeats the two elements do not come into contact (as corroborated by Egelhaaf (1989); Tu and Dickinson (1994, 1996); Balint and Dickinson (2001)).

Despite this, Nalbach claims that the structures to engage the wing must be pulled medially in order to engage, and suggests that the muscles of *Ax1* or *Ax3* may be suitable. She observed that the *Ax3* complex always moves during switching and indeed Walker et al. (Walker et al., 2012) find that movements of *Ax3* control movements of the alula, which is itself possibly associated with gear changes. Furthermore, Balint & Dickinson (Balint and Dickinson, 2001) suggested that *III2-4* activity prevents *RS-PWP* engagement due to their effects on wingbeat amplitude. *Ax1*, also appears to play some role in regulating wing kinematics as Balint & Dickinson (Balint and Dickinson, 2001) found that the forward amplitude of each

wingbeat was reduced whenever there was activity in *Ax1* muscles (also found by Walker et al. (2014)). Later studies (Balint and Dickinson, 2004) also found changes to other kinematics such as stroke deviation and the timing of wing rotation, all of which are associated with the gear change mechanism. *Ax1*, *Ax3* and the basalar sclerite all therefore seem to be candidates for the control of the mechanism.

2.6.5 Flight Control Musculature: Conclusions

The indirect power muscles in dipteran insects are poorly situated to induce changes in wing kinematics, particularly bilateral asymmetries (but see Lehmann et al. (2013)). Instead, it is the direct control muscles (the steering muscles) that are primarily recruited to induce asymmetrical changes in wing kinematics. The sclerites to which many of these muscles attach are integral to the wing hinge itself, and so any change in their position will cause a great degree of change in the movement of the wing. Indeed, the many studies which have examined the activity of these muscles have found concurrent changes in wing kinematics, but not the biomechanics through which this is brought about.

The basalar sclerite is the best studied sclerite-muscle complex, and so we have a good understanding of its function and the mechanism through which this is achieved. *b1* has been shown to perform negative work (Tu and Dickinson, 1994), thereby acting to inhibit the movement of the basalar sclerite, while the other muscles change the position and movement of the sclerite during the wingbeat. Since this sclerite is connected to the wing via a ligament, these muscle activity-induced changes directly influence wing movement. The negative work performed by *b1* (and possibly other basalar muscles) allows it to do work more effectively and efficiently than if it were performing positive work. This is a very important feature for muscles which are functioning in an oscillating system working against the positive work of power muscles which are much larger than themselves. For this reason, it may be a feature shared by other steering muscles.

Negative work has the added benefit of being possible without a muscle twitch

duration that is fast enough to allow the muscle to undergo a contraction cycle at wingbeat frequency. This would be metabolically expensive and would require a large amount of muscle volume to be occupied by sarcoplasmic reticulum (which is required for the storage, release and reuptake of calcium during contraction and relaxation cycles), thereby reducing the volume of contractile myofibrils within the muscle and decreasing the possible force output of the muscle. The large buckling tendons of *I1* and *I2* muscles permit a large range of sclerite movement even when the muscles are fully contracted, subverting the requirement of these muscles to undergo contraction cycles at wingbeat frequency. Whether these and other muscles also act through negative work like *b1* requires confirmation in future work. Studies of insects which operate at lower wingbeat frequencies are also warranted to determine whether their control muscles also show the same adaptations, or whether brachyceran insects like *Calliphora* and *Drosophila* are constrained to show certain features due to their rapid wing movements.

The muscles of the basalar sclerite also illustrate another quality of the control muscle complex, in that the muscles attaching to the same sclerite often bring about the same changes in wing kinematics, posing the question of why the same job could not be performed by a single muscle. However, as shown by Lindsay et al. (Lindsay et al., 2017), the muscles can be grouped into small, frequently active muscles influencing fine kinematic control, and large, infrequently active muscles affording coarse kinematic control. Each sclerite has at least one muscle of each type attached to it, giving dipteran insects precise control over the movement of the wing hinge sclerites, and the movement of the wing as a result.

There is still much to learn about this remarkably complex system, and fortunately novel methods are allowing us to visualise the system in new ways, and begin to tease apart the biomechanics of these muscles (e.g. Walker et al. (2014)). Chapter 7 uses a combination of time-resolved synchrotron X-ray μ CT and high speed videography to study the motion of these muscles within living insects, and correlate changes in muscle strain patterns to changes in wing kinematics. I then

use this knowledge to suggest mechanistic explanations for how exactly these muscles influence the movement of the sclerites to which they attach, and in doing so determine the kinematics of the wings.

2.7 Discussion

A recurring theme throughout this review is the resistance of motion, both of the wing hinge components and of the wing itself. The muscles of the basalar sclerite are thought to act as variable tension-springs absorbing energy from the movement of the sclerite to influence wing movement (section 2.6), and this may be a feature shared by other control muscles (section 2.6). The interaction between the *RS* and the *PWP* that forms the gear change mechanism is also thought to act as a brake-like mechanism, absorbing energy from the wing to determine its kinematics during the downstroke (section 2.4). Finally, even the contact between the *PtC* and the *RS* at the end of the downstroke may be another example of using energy absorption to limit the movement of the wing (section 2.5). This restriction of wing movement is the mechanism through which these various components of the wing hinge determine wing kinematics and govern flight.

The power muscles do not alter their activity during these manoeuvres, meaning that some of the force normally translated into wing movement through the wing hinge is seemingly not utilised or wasted when the movement of the wings is inhibited to generate whole-body torques. That is to say that the various mechanisms used to control wing kinematics appear to do so by working against the power muscles themselves. However, considering the enormous size of the power muscles relative to the body and the rest of the flight motor components (occupying $92.3 \pm 1\%$, of the total musculature within the thorax in ten of our specimens, mean $\pm$ one standard deviation), it may not be possible to substantially increase the amount of power supplied for wing movement using any other component. Instead, the various components of the flight motor may be better used to absorb some of this force from the power muscles, or manipulate the way in which it is translated through the wing

hinge to the wing such that wing kinematics are altered.

Furthermore, it may be the case that the large amount of energy absorbed by these different mechanisms is not lost from the system, but is instead stored and used to help drive movement throughout the next half-stroke and drive the system at resonance. Indeed, the dipteran flight motor is thought to be heavily reliant on resonance to maintain efficiency within the system (Roeder (1951); Greenewalt (1960); Dickinson and Lighton (1995); Brennan et al. (2003); Ha et al. (2013); Deora et al. (2017) though see Ramananarivo et al. (2011)). In which case, kinetic energy absorbed by the *RS-PWP* interaction during the downstroke may be stored as elastic energy which is then used to drive wing movement at the start of the upstroke. This would mean that energy is not wasted, and is instead just reallocated.

The dipteran flight motor very effectively makes use of a regular, repeatable force generated by the power muscles and uses this to generate a variety of wing kinematics by changing the ways in which this force is translated into wing movement. This occurs, for example, by the negative work of at least some of the control muscles (as has been identified in *b1*) inhibiting the motion of wing hinge components, or the gear change components directly preventing the movement of the wings. The energy stored as elastic energy through this action may then be used to overcome the inertial forces required to reverse the wing's path at the end of each half-stroke, which can stem from both the wing's mass and the added mass of the air which must be accelerated. This same energy could also be used to ensure the system continues to function at resonance by changing wingbeat frequency, for example.

If the flight motor of dipteran insects does indeed function through the extensive use of negative work this would suggest that these animals control their wing movements using mechanisms which are fundamentally different to most artificial designs. This thesis explores the various aspects of the flight motor described in this review. The findings of the studies outlined here reveal evidence for the widespread utilisation of negative work and energy absorption within the dipteran flight motor. These same conclusions may apply to the control of wing kinematics in other insect

orders, and this possibility merits further research. Biomechanical studies such as this are of interest not only to biologists and entomologists, but also to engineers designing highly efficient mechanisms or vehicles which take inspiration from the systems found in nature.

2.8 Conclusions

Dipteran insects are some of the most aerobatic species on the planet, and so it is no surprise that they possess myriad mechanisms of wing kinematic control. The power muscles are the source of the gross wing motion during the wingbeat, the gear change mechanism provides coarse control over wing motion (and the *PtC* may have some influence too), while the control muscles that attach to the various components that make up the wing hinge afford finer control over wing kinematics. This review has made use of synchrotron X-ray μ CT data to illustrate these mechanisms and the anatomy of the dipteran flight motor in great detail, and has also highlighted the potential importance of energy absorption and the inhibition of component motion within the system.

In doing so, this work has also illustrated how much there is still to learn about the dipteran flight motor. Despite the large kinematic changes associated with changes in the state of the gear change mechanism, whether these alterations are caused by the direct inhibition of wing movement by the interaction, or whether the engagement enables wing kinematic control through other means, is still a matter of debate. Similarly, the mechanisms through which the control muscles bring about their observed influences on wing motion are also not fully understood. The enigmatic *PtC* has also received comparatively little attention given its prominence within the wing hinge, and its role within the flight motor therefore remains unconfirmed.

This remainder of this thesis examines each of these aspects of the flight motor in turn using experimental methods; examining the biomechanics through which they afford Diptera such remarkable flight capabilities. In Chapter 3 I examine the gear

change mechanism in *Calliphora vicina* using high speed macrophotography and three dimensional photogrammetry to answer the long standing questions regarding the timing of *RS-PWP* interaction and its effects on wing kinematics. The following chapter uses these same techniques to examine the gear change mechanism in another species, the drone fly *Eristalis tenax*, to identify any differences in the mechanism between species. Chapter 5 then amalgamates the findings of these previous two chapters, as well as studying the components of the mechanism in more detail using video tracking and ablation experiments. In doing this, these three chapters build on previous hypotheses of the interaction acting as a brake or wing rotation aid, and provide new insights into its biomechanics and its role within the dipteran flight motor.

Chapter 6 rounds off the study of the external anatomy of the wing hinge by using macrophotography to examine the role of the Pterale C within the flight motor. Using macrophotography, we document the interaction of this component with the *RS* and in doing so provide a novel insight into the role of the *PtC*, contributing to the long standing debates over its potential function as either a proprioceptor or a mechanical stop.

Finally, Chapter 7 delves into the internal anatomy of the wing hinge of *C. vicina* using time resolved synchrotron X-ray computed μ CT. With this technique, we study the movement and strain patterns of three muscles of the particular importance within the flight motor, *I1*, *I2* and *b1*. Using these data, we are able to correlate bilateral differences in muscle strain with bilateral asymmetries in wing kinematics and in doing so elucidate the biomechanics through which these muscles bring about the wing kinematic changes observed both in this study and in those of other authors studying these muscles.

Gaining a detailed understanding of each of these components of the flight motor is essential if we are to be able to translate the biomechanics of this system into the design of successful, bio-inspired flapping wing MAVs; and this thesis represents significant progress towards this ability.

Appendices

Appendix A

Methodologies and Techniques

A.1 Radiograph Acquisition

We carried out our experiments at the Swiss Light Source facility (Paul Scherrer Institut, Villigen, Switzerland), using the TOMCAT beamline to acquire time-resolved microtomographic images of the thorax of several *C. vicina* individuals (figure 2.3; Stampanoni et al. (2010); Walker et al. (2014)). The X-rays required for radiograph production were produced by a superconducting bending magnet 25 metres from the insect. A multilayer monochromator made of alternating Ruthenium and Carbon layers placed 7 metres downstream of the source monochromatised the beam to an energy of 16keV and a bandwidth of approximately 2% to achieve a high X-ray flux. The beam height was 4.893mm, with a width of 5.402mm.

To tether the insect in the path of the beam, we first cold-anaesthetised the individuals at 3-5°C for between 5-10 minutes, depending on the size of the insect, until it was completely immobile. We then placed the insect in a groove cut out of a sponge, and adhered a plastic mount to the scutum with a warmed mixture of beeswax and colophonium. Once cooled, this mixture strongly adhered the insect to the tethering mount. We then placed the tether in the path of the beamline ahead of a 100µm Ce-doped LuAG scintillator positioned 320mm away. This scintillator detects the X-ray radiation and converts it into visible light. This light image was then magnified by a factor of between 2 and 4 using a custom-built high numeri-

cal aperture optical microscope (Elya solutions, s.r.o). Finally, these images were recorded using TOMCAT's GigaFRoST detector (Mokso et al., 2017). We used a magnification factor of 2.25, and given the physical pixel size of 11 μm on the GigaFRoST detector, the effective pixel size for the radiographic projections is 4.893 μm .

While the insect was flying we rotated the insect at 90°s^{-1} during the scan, while the GigaFRoST recorded at 3934.28 Hz with an exposure time of 0.25ms. A full 360° scan of the insect therefore took 4 seconds, resulting in around 15,737 projection images.

After we acquired the time-resolved radiographs, the insects returned to a resting position with their wings folded over the back. This allowed us to collect a further scan in which the muscles were at their assumed resting length. We thereby performed a more detailed scan of the insect at a rotation speed of $11.995^\circ\text{s}^{-1}$ with a 10ms exposure time, which produced 1,500 projection images over its 180° rotation.

A.2 Tomographic Reconstruction

After radiograph acquisition, we corrected the images using flat field correction, making use of flat-beam images (images taken with no sample) and dark images (images taken with no beam) collected immediately after the scan. We determined the phase of each radiograph in a qualitative manner using ANKAphase implementation, assuming that the object consisted of a homogeneous soft tissue material (Paganin et al., 2002; Weitkamp et al., 2011) and that the steering muscles had a refractive index equal to that of water (Henke et al., 1993). The real and imaginary parts of the deviation from the complex refractive index of the material were 1×10^{-10} and 1×10^{-6} , respectively. The reconstruction of the radiographs was performed using a custom written MATLAB (The Mathworks Inc., Natick, MA, USA) script which implemented a Fourier transform-based algorithm (Marone et al., 2010). The voxels produced during these reconstructions had an isotropic spacing of 3.3 μm .

A.3 Three-Dimensional Wing Kinematic Resolution

We positioned two Photron AX200 FASTCAM Mini cameras (Photron Ltd., West Wycombe, Buckinghamshire, UK) above the insect rotation platform, such that both the wings of the insect would be in view of both cameras. To ensure the insects were adequately illuminated, we used a custom built ring of 9 infrared LEDs to backlight the insect (figure A.1), as insects are insensitive to wavelengths in the IR spectrum (Smola and Meffert, 1979; Tinbergen and Abeln, 1983; Lunau, 2014). We fitted a 3mm layer of semitransparent acrylic above the LED ring to diffuse the illumination equally across the camera frame. To film the wings with appropriate clarity, we set the cameras to film at 4,000 frames per second, with a shutter speed of $10.67\mu\text{s}$. We also cut a small groove into the light diffuser acrylic into which we slotted the base of the insect's tether to ensure the insect was in a consistent position throughout the filming.

The calibration procedure used in this study to obtain the camera parameters required for photogrammetric reconstruction of the wing position was based on the same procedure used by Walker et al. (Walker et al., 2010). This method uses a custom-written MATLAB script implementing a bundle adjustment procedure to produce estimates of the camera parameters from synchronised images of a small calibration grid. We then used another script to track the wingtips in three dimensions across the course of the wingbeat (figure A.1).

A.4 Time Resolution of Radiographs

Each recording consisted of approximately 1200 wingbeats, during which time the insect was being gradually rotated on a stage within the patterned cylinder (as seen in figure 2.3). We used the 3D wingtip positions determined from the synchronised cameras to identify individual wingbeats, starting at the beginning of the downstroke, which were then interpolated to give twenty evenly spaced time points. We

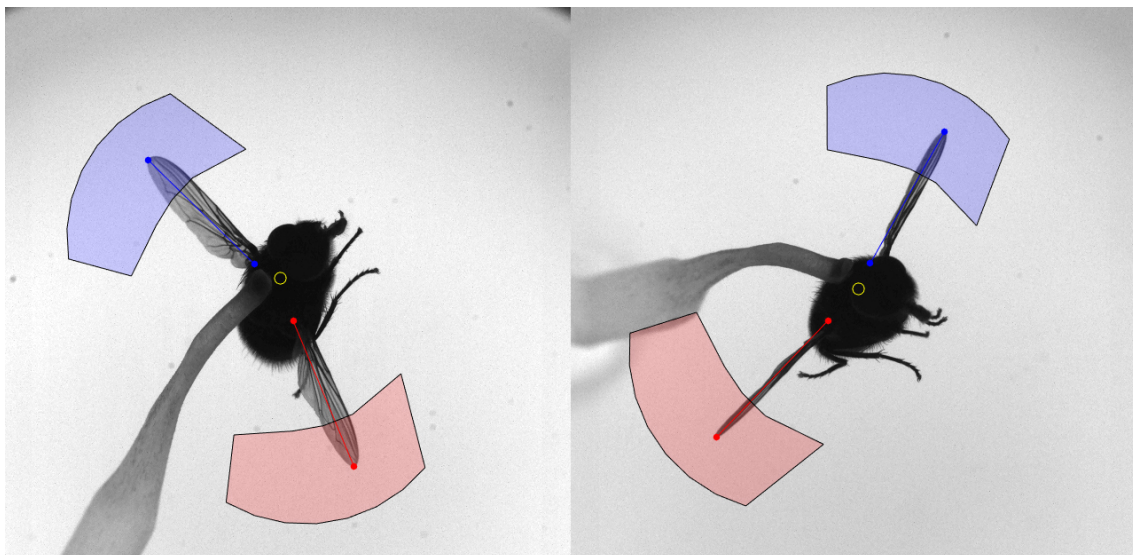


Figure A.1: Video frames illustrating the collection of three-dimensional wing kinematics using two synchronised cameras. Blue and red dots indicate the left and right wings, respectively, and the lines leading to these points from the wing base are equal to the wing length. The red and blue patches indicate the cropped area of the frame in which the tracking algorithm will search for the wingtip (the farthest point in the outline of the wing obtained through background subtraction) in the next frame. The yellow circle indicates the centre of rotation of the insect during radiograph recording. The plastic rod to which the insect is mounted can also be seen in the both images.

then used the time stamps to find the closest matching radiograph for each time point in every wingbeat. This resulted in twenty sets of radiographs where each set consisted of around 1200 images of the insect captured at the same stage of the wingbeat cycle, but in a different orientation. These could then be treated as a collection of static scans and a 3D reconstruction performed on each set to give a full four-dimensional composite wingbeat.

A.5 SEM Image Collection

We took individuals of both *C. vicina* and froze them before air-drying the specimens. The wings of the insect were often naturally resting upwards such that the wing hinge was revealed. If this was not the case, though, we manually manipulated the wing into the appropriate position, taking care not to damage the wing or any other surrounding area. We then coated the insects in an Au-Pd alloy using an SC7620 sputter coater (Quorum Technologies, UK) for 150 seconds to give a 7nm coating. Finally, making use of a JCM-5000 NeoScope (Jeol, Japan) scanning

electron microscope, we collected images of the right wing hinge.

Chapter 3

The Gear Change Mechanism in *Calliphora vicina*

Dipteran insects are able to control their wing movements with extreme precision, and make alterations to wing kinematics on a wingbeat-by-wingbeat basis. One mechanism thought to be important in this control is the gear change mechanism: an interaction between the radial stop (*RS*) on the underside of the wing, and the pleural wing process (*PWP*) that projects from the body just underneath the wing hinge. The *RS* can engage with one of several grooves on the *PWP*, and wingbeats can be classed as being in different ‘gears’ depending on whether this occurs. The precise result of the engagement, however, and its timing within the wingbeat, has remained a matter of debate. Previous authors have also postulated that a movement of a proximal portion of the wing, the alula, may be related to the gear change mechanism, and indirectly act as an indicator of the engagement of the two sclerites. Here, we use high-speed macrography to film the interaction, and document the alula moving between different states. We also make use of photogrammetry to identify three-dimensional wing kinematic changes across wingbeats engaged in different gears. We find that the alula can occupy different positions regardless of the type of *RS-PWP* interaction, but shows a propensity to occupy certain states in different gears, meaning that it can be used as a rough indicator of the state of

the gear change mechanism. There are also significant relationships between *RS-PWP* interaction and every wing kinematic parameter measured, suggesting that the gear change mechanism plays an important role in the control of dipteran wing movements.

3.1 Introduction

Diptera are amongst some of the most manoeuvrable flying insects, a group which is already renowned for its flight capability. Many attribute this to their unique hind-wing halteres (Pringle, 1948; Hengstenberg et al., 1986; Nalbach, 1993; Fayyazuddin and Dickinson, 1996; Daniel et al., 2012; Parween and Pratap, 2015; Agrawal et al., 2017), which affords agility through detailed and rapid sensory feedback during flight.

The flight motor itself, however, must provide the physical components that actually allow the wings to rapidly change their patterns of movement in response to the sensory information provided by the halteres. These changes in wing motion are brought about through the highly complex flight control musculature that attaches to and around the elements of the wing hinge (e.g. Heide (1983); Wisser and Nachtigall (1984); Tu and Dickinson (1996); Dickinson and Tu (1997); Balint and Dickinson (2004); Walker et al. (2014); Lindsay et al. (2017)). Despite their small size, these muscles make use of mechanisms to amplify the effects of their force output and expend energy efficiently.

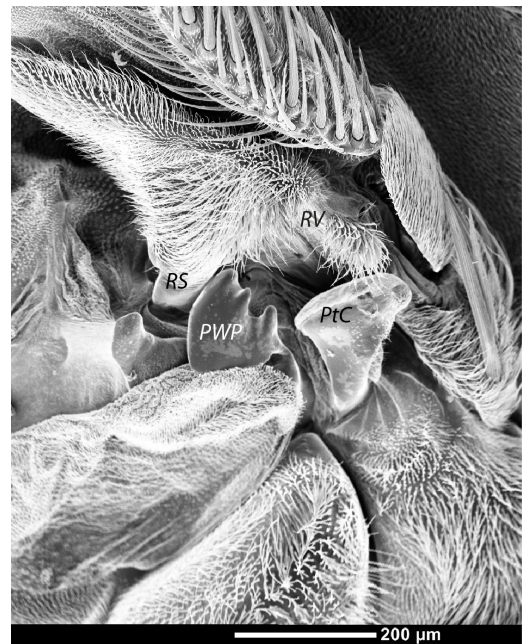


Figure 3.1: An SEM image of the right wing hinge of *Calliphora vicina* (anterior is to the right). The *RS* is locked behind the *PWP* in a gear 0 position. Note the two separate grooves in the top of the *PWP*. Abbreviations: *PWP* - Pleural Wing Process; *PtC* - Pterale C; *RS* - Radial Stop; *RV* - Radial Vein of the Wing.

One proposed system is the gear change mechanism, which involves the inter-

action of two sclerites. The first, the radial stop (*RS*), is a tooth-like outgrowth found on the underside of the radial vein of the wing. The second, the pleural wing process (*PWP*), is a projection from the pleural wall with several grooves found on the dorsal edge of the head (figure 3.1; Pfau (1973); Miyan and Ewing (1985a)). The *RS* is able to lock into each of the grooves of the *PWP* during the wingbeat (figure 3.2), with the resulting interactions, termed ‘gears’, creating significant changes to the wing movement. One extra interaction, termed ‘gear 0’, occurs as the *RS* locks behind the rear shoulder of the *PWP*. Though gear 0 completely inhibits the wingbeat, it does permit wing vibration, as occurs during courtship song production in some species (Bennet-Clark and Ewing, 1968; Chowdhury and Parr, 1981).

These interactions between the *RS* and *PWP* have been examined by a number of different authors (Pfau, 1973; Miyan and Ewing, 1985a; Pfau, 1987; Wisser, 1988; Nalbach, 1989; Balint and Dickinson, 2001; Walker et al., 2012; Deora et al., 2015), but the precise mechanism and the results of the interaction itself have been a topic of argument. As a result, a conclusive description of its functioning has not yet been produced. The two structures vary in shape and size across species, particularly the *PWP* (Miyan and Ewing, 1984, 1985a), which is heart shaped in *Calliphora* with two grooves, while in *Drosophila* it is square with a single groove. Given that the number of grooves determines the amount of possible *RS-PWP* interactions, and thereby possibly the resolution of wing kinematic control the mechanism can exert, these components have clearly been adapted for different roles and must have an important function.

Part of the reason it has evaded complete description is because the mechanism is difficult to study. Classical techniques such as stroboscopic illumination (Wisser, 1988; Nalbach, 1989) are useful, but limited in their ability to study small components undergoing such high frequency movements as those observed during dipteran flight. With the advent of new techniques however, such as high speed macrography, more detailed examination of this mechanism has become possible.

One study of drone flies (*Eristalis tenax*), which utilised high speed cameras to

film the movement of the wings during free flight manoeuvres in detail also revealed the unexpected behaviour of a proximal component of the wing known as the alula (figure 3.2; Walker et al. (2012)). The alula is a proximal component of the wing which is capable of occupying either a ‘flat’ position lying parallel to the rest of the wing, or a ‘flipped’ state in which the alula is perpendicular to it (Lowne, 1890; Colyer and Hammond, 1968; Weis-Fogh, 1973; Stubbs and Falk, 2002; Starý, 2008). As the alula moved position, Walker et al. recorded associated wing kinematic changes that were reminiscent of the effects of the gear change mechanism. This led to their hypothesis that alula-flipping is in some way linked to the gear change mechanism, meaning that the two may share the same underlying muscular control.

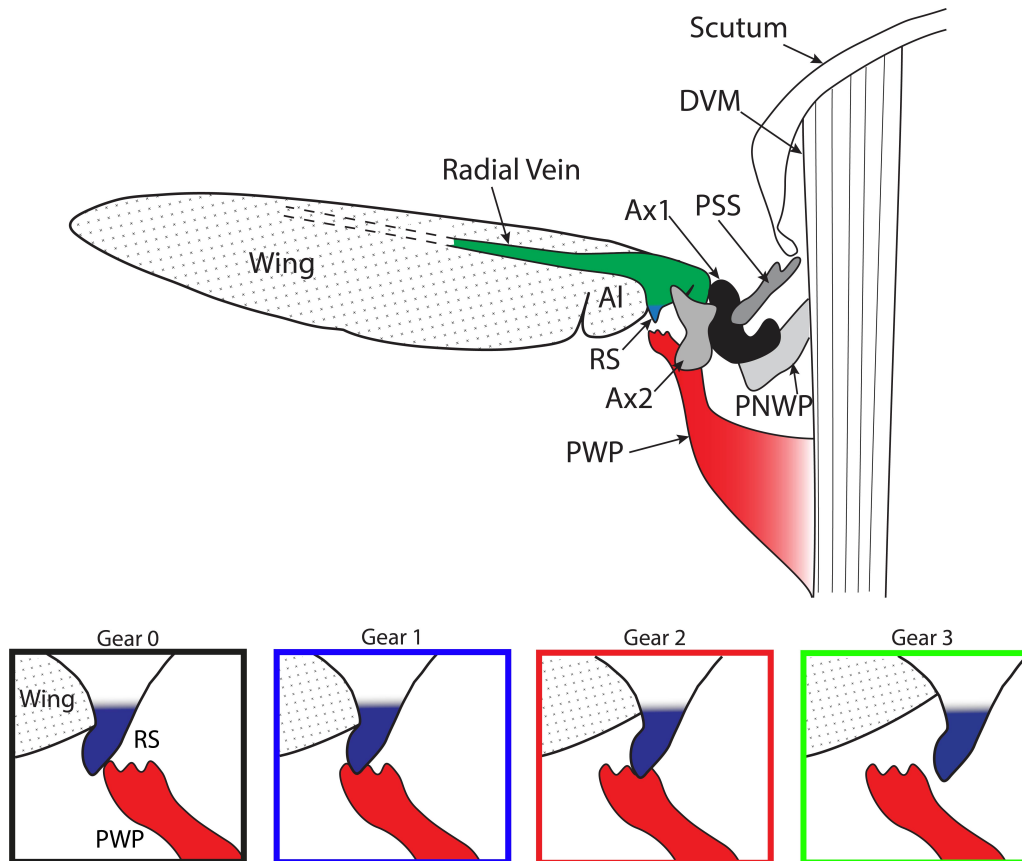


Figure 3.2: A simplified schematic of the cross section of an *C. vicina* thorax and wing hinge, illustrating how the *RS* and *PWP* can interact during the end of the downstroke in the gear change mechanism. During gear 0, the *RS* locks behind the posterior shoulder of the *PWP* and severely inhibits wing motion. In gears 1 and 2, the *RS* engages with the grooves of the *PWP*, while in gear 3 there is no interaction and the *RS* passes anteriorly of the *PWP*. The number of grooves in the *PWP* is thought to determine the number of gears. Abbreviations: *Al* - Alula; *Ax1* - First axillary sclerite; *Ax2* - Second axillary sclerite; *DVM* - Dorsoventral muscles; *PNWP* - Posterior notal wing process; *PSS* - Parascutal shelf; *PWP* - Pleural Wing Process; *RS* - Radial Stop. Modified from Walker et al. (2012).

Studying this relationship further could therefore help us understand how these components of the flight motor are controlled and give insights into how gear changes are used during free-flight.

Using high-speed macrography we have documented this alula movement and the gear change mechanism during tethered flight of the blow fly, *Calliphora vicina*. Making use of photogrammetric techniques, we have also been able to quantify wing kinematics, and identify the differences in these movements across wingbeats undergoing the various types of *RS-PWP*. This has enabled us to answer many of the long standing questions regarding the timing of this interaction during the wingbeat, and its effects on wing kinematics. We find that wingbeats during which the two components of the gear change mechanism interact exhibit significantly different wing kinematics when compared to wingbeats in which there is no interaction. Furthermore, these kinematics also differ significantly depending on the position of the alula, but we fail to find any definitive relationship between the movement of the alula during different gears. We discuss how *RS-PWP* interaction may function to bring about these changes in wing kinematics, and how these may be utilised to control whole body-manoeuvres during flight. Improving our understanding of these unique mechanisms in dipteran insects will enable us to apply the same engineering principles to micro- or nano-machine designs.

3.2 Materials and Methods

3.2.1 Insect Preparation

We purchased *C. vicina* larvae from a local retailer and incubated them within a lab at room temperature (20-22°C) until pupation and hatching. During the larval stage, we provided the insects with strips of beef, and placed a damp paper towel within the enclosure. Once the larvae had pupated, we removed any remaining food and continued to provide damp paper towels.

Adults typically emerged after around two weeks of room temperature incubation

into an insect rearing tent (Watkins and Doncaster Bugdorm-43030). Throughout the lifespan of the adults, we provided them with a purée of banana and infant nutritional supplement powder once every two days as a source of nutrition, along with continued provision of damp paper towels as a water source.

We found that the cuticle of the adults had not fully hardened until around 48 hours after hatching. We therefore avoided utilising the insects in tethered flight until after this time.

Before tethering, we cold anaesthetised the insects at between 3-5°C for 5-10 minutes depending on the size of the insect, until it was completely immobilised, and placed it in a small groove cut into a sponge. We then placed a small amount of a heated beeswax and colophonium mixture onto the scutum of the insect, and lightly pressed a metal rod against this to tether the insect.

We then secured the base of the tether rod into a manual three-axis linear motion stage in the center of the camera set up to position the insect in view of the cameras. Before and between bouts of filming, we placed a small wetted ball of tissue under the insect to hydrate and cool the animal.

3.2.2 High-Speed Camera Set Up

We positioned four high-speed cameras (two Photron FASTCAM Mini AX100 type 800K-M-16G cameras, and two Photron FASTCAM SA3 model 120K-M2 cameras, Photron Ltd.) around the insect to film both the wings and the wing hinge in detail. Using two supporting poles, we positioned the two SA3 cameras above the insect such that the left wing was visible for the entirety of the wingbeat. We then placed one Mini AX100 on a tripod behind the insect to view the wing from a posterior angle. Finally, to film the wing hinge, we fitted a second AX100 with 190mm of extension tubes and bellows to achieve a 4:1 magnification ratio. We positioned the camera view onto a gold-plated mirror (as gold plating reflects infrared light more effectively than a non-plated mirror), which itself was angled such that it reflected an image of the insect's left wing hinge from a lateral and slightly anterior viewpoint.

To film the wing with appropriate resolution and clarity, we set the three cameras filming the wing to record at 3,200 frames per second (fps), with a shutter speed of 33.3 μ s. For the camera filming the wing hinge, we set a higher frame rate of 6,400 fps (synchronised so that every other frame had the same time stamp as the other cameras) to view the movements of the small components in more detail, with a shutter speed of 100 μ s.

Due to the rapid shutter speeds of the cameras, we used two custom-built high-power infrared (IR) LEDs to illuminate both the wings and the wing hinge. These LEDs emitted light with a peak intensity at a wavelength of 860nm, and this reduced to zero by 760nm. These insects however are insensitive to wavelengths of above around 600nm (Smola and Meffert, 1979; Tinbergen and Abeln, 1983; Lunau, 2014), meaning that the insects were not able to see the light radiating from the LEDs. To focus the light onto the desired area, we also attached two plano-convex lenses (Edmund Optics Ltd.) onto one of the LEDs to focus and subsequently collimate the light to illuminate the wing hinge. On the second LED, we placed one plano-convex lens to collimate the light and illuminate the whole wing across the duration of the wingbeat.

These lights generated a large amount of heat directed towards the fly, so we ensured that we disabled the lights whenever they were not necessary. The lights were never directed onto the fly for more than ten seconds at a time. Furthermore, to avoid the fly overheating during flight, we placed a small fan above the insect and connected it to a container filled with ice. This therefore provided a humidified airstream which both cooled the insect and provided a stimulus for flight.

3.2.3 Three Dimensional Wing Kinematics Measurement

The calibration procedure used in this study to obtain the camera parameters required for photogrammetric reconstruction of the wing position was based on the same procedure used by Walker et al. (Walker et al., 2010). This method uses a custom-written MATLAB script implementing a bundle adjustment procedure to

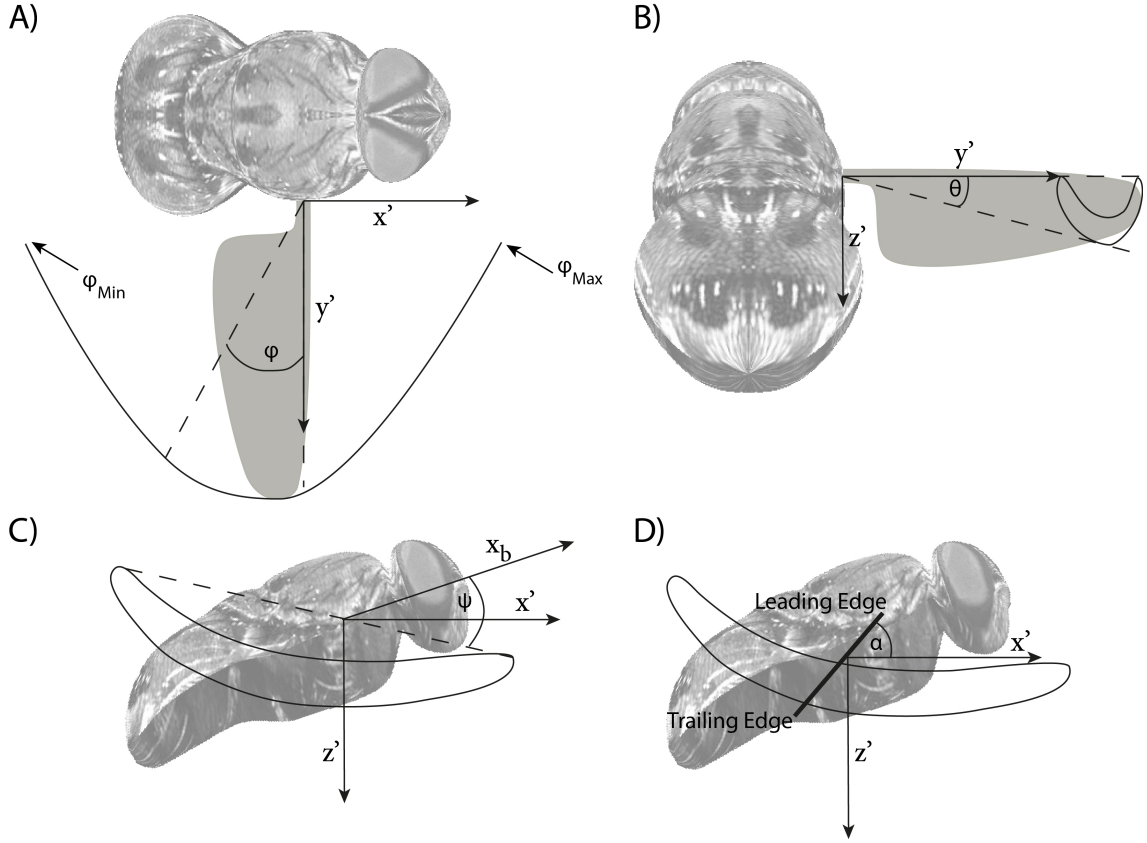


Figure 3.3: Definition sketches of the measured wing kinematics. Measurements are made in a right-angled, body-fixed axis system (x', y', z') with an origin at the wing's base. The $x'y'$ -plane is defined by the stroke plane, which itself is defined as the plane connecting the wing base with the positions of the wingtip at the end of the upstroke and at the bottom of the downstroke. The x' -axis lies on the stroke plane and is parallel to the insect's sagittal plane, oriented forwards. The y' -axis again lies on the stroke plane, normal to the x' -axis, and points towards the right side of the insect's body. Finally, the z' -axis is perpendicular to both these axes, oriented downwards. (A) The stroke angle (ϕ) is defined as the angle between the line joining the wing tip and the wing base, and its projection onto the stroke plane (or the $x'y'$ -plane). Stroke angle is positive anterior of the wing base, and negative posterior to it. (B) The deviation angle (θ) is measured orthogonal to the stroke angle, being defined as the angle between the line joining the wing tip and the wing base and its projection onto the $x'y'$ -plane. Stroke deviation angle is positive above the $x'y'$ -plane and negative below it. (C) The stroke plane angle (ψ) is defined as the inclination of the plane relative to the body's sagittal plane (x_b). Here the curved line shows the wingtip path, while the horizontal dashed line shows the stroke plane. The angle of this line projecting onto the $x'z'$ -plane gives the stroke plane angle. (D) The wing pitch angle (α) is defined by the angle of the line connecting the leading and trailing edges of the wing relative to the stroke plane.

produce estimates of the camera parameters from synchronised images of a small calibration grid. We then used another script to track the shape and position of the wing in three dimensions across the course of the wingbeat using a voxel-carving algorithm. The script was also able to fit splines to the leading and trailing edge of the wing outline, enabling us to determine the relative positions of the two. We filtered the data using a quintic smoothing spline which used a tolerance factor calculated from the residuals resulting from fitting a third-order Butterworth filter to the data. This filter used sampling and cut off frequencies of 3,200 Hz and 800 Hz, respectively. We also tested a range of other cut off frequencies (800 ± 400 Hz) and found no significant effect on the results presented here. Finally, we used cubic interpolation to estimate the three-dimensional wingtip position at 101 evenly spaced timesteps for each wingbeat. From these data we could summarise the position of the wing in terms of the stroke angle, stroke deviation angle, stroke plane angle, and wing pitch angle (figure 3.3).

All wing kinematic parameters were measured with respect to the stroke plane. The stroke plane is defined for each half stroke (each of the upstroke and downstroke), as the plane formed by the wing base and the position of the wing tip at each stroke reversal. The angle of this stroke plane is then defined as the angle of the plane relative to the long axis of the body, which is negative when it is tilted nose-down.

The wingtip itself is then defined by a pair of spherical coordinates, the stroke angle and the deviation angle. The stroke angle is measured within the stroke plane and is positive when the wingtip is anterior to the wing base. The deviation angle is then measured normal to the stroke plane, positive while the wingtip is dorsal to it, and by definition is zero at the beginning of each half stroke. The wing pitch angle is measured at the mid-length of the wing, and measured as the angle of the line that connects the leading edge and the trailing edge half-way along the wing relative to the horizontal.

Stroke amplitude is defined as the absolute difference in stroke angle between the

beginning and end of the downstroke, while stroke deviation range is defined as the range between the maximum and minimum deviation angles across the wingbeat. The maximum and minimum values of each kinematic parameter are extracted from the entire wingbeat, including both the upstroke and downstroke, unless specified otherwise. In the case of stroke angle, the minimum stroke angle occurs at the start of the downstroke, while the maximum stroke angle occurs at the end of the downstroke. We also calculated the timing of wing supination, defined here as the time at which the wing pitch angle increases from less than 90° during the downstroke to greater than 90° as the wing reaches the end of the downstroke. Finally, we calculated the wingbeat frequency and the timing of the end of the downstroke.

3.2.4 Wingbeat Classifications

We used the high speed recordings of the wing base to classify each wingbeat as gear 0, 1, 2 or 3 depending on whether and how the *RS* and *PWP* interacted during the wingbeat. We defined gear 3 as a wingbeat in which the *RS* and *PWP* did not come into contact at all, and defined gears 2 and 1 as wingbeats in which the *RS* contacted the anterior and posterior grooves, respectively (figure 3.2). Finally, we defined gear 0 as a wingbeat in which the *RS* locked behind the *PWP*, such that the *RS* never moved dorsally of the *PWP* as it does in other gears. This was a rarely engaged gear in energetic flights, and we excluded flights dominated by these wingbeats from our final analyses.

We examined the wing using the three views of the whole wing to identify whether or not the alula was flat or flipped. This was generally clearest at the point of supination at the end of the downstroke. If the alula was flat to the wing, it was clearly visible, whereas it was not visible if it was flipped (figure 3.4).

3.2.5 SEM Image Collection

We collected individuals of *C. vicina* and froze them before air-drying the specimens. We then undertook the same methodology as outlined in Chapter A.1.

3.2.6 Statistical Analysis

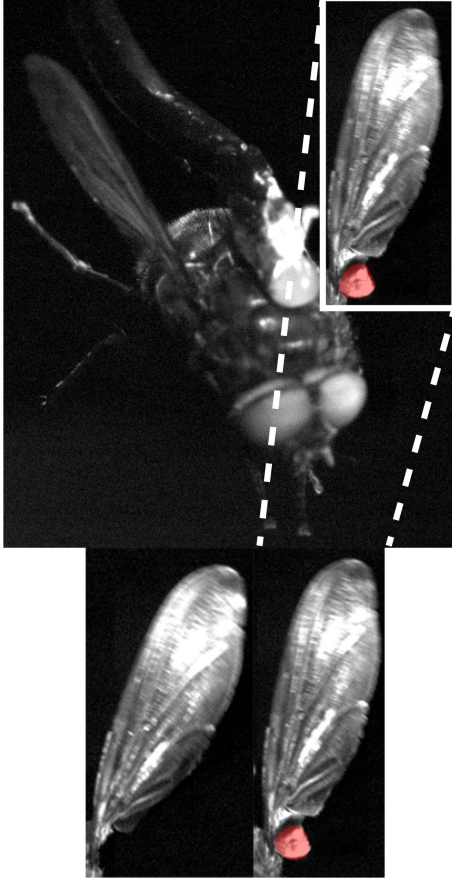


Figure 3.4: An image of a *C. vicina* individual in tethered flight, highlighting the the wing with a flat alula (highlighted in red). The bottom panels show the wing with the alula in both a flipped state (left), where the alula is not visible, and the alula in a flat state (right) where the alula is clearly visible at the base of the wing proximal to the hinge.

To determine statistically significant differences amongst the kinematics of wingbeats in different gears, we used one-way analysis of variance (ANOVA) tests. Further to this, we utilised Tukey-Kramer tests to identify significant differences between pairs of gears. As there are only two alula states, we performed two-sample t-tests to identify differences in kinematics between wingbeats exhibiting different alula states.

After classing each wingbeat according to its gear engagement and alula state, we used χ^2 tests to observe any significant relationship between alula-flipping behaviour and *RS-PWP* interaction. To determine where any significant relationships stemmed from within the contingency table of gear engagement and alula states, we calculated ad-

justed residuals (r_{adj}) between the expected and observed values using the following equation:

$$r_{adj} = \frac{O - E}{\sqrt{E \times (1 - \frac{Rt}{n}) \times (1 - \frac{Ct}{n})}} \quad (3.1)$$

Where O is the observed value, E is the expected value, Rt is the row total for that alula state, Ct is the column total for that gear state, and n is the total number of observations.

Finally, we utilised a ‘ransacking’ method (Sharpe, 2015), in which we performed separate χ^2 tests on each of the 2-by-2 contingency tables within our original 2-by-3 contingency table of alula state versus gear engaged. This enabled us to highlight any significant differences in alula states between pairs of gears, and thereby determine whether individual gears can be identified on the basis of whether or not the alula is flipped. We performed all our statistical analyses and data manipulation using MATLAB (The Mathworks Inc., Natick, MA, USA).

Throughout these analyses, we applied a false-discovery rate algorithm to each individual set of comparisons (the across-gear state and across-alula state kinematic comparisons are treated as separate for this purpose) to compensate for the large number of tests being performed and reduce the risk of type I errors. To do this, we ranked the p -values from each set of analyses in ascending order (i.e. the most significant results ranking first), and compared this value against an adjusted critical α -value, defined as 0.05 divided by the rank of the p -value. For example, the most significant value is compared against a critical value of 0.05, while the second is compared against a value of $0.05/2 = 0.025$, the third against $0.05/3 = 0.017$, and so on. Provided the p -value is smaller than the adjusted critical α value, the null hypothesis is rejected. Once we encounter a comparison with a p -value that is greater than the adjusted critical α value, we conclude that we cannot reject the null hypothesis of this comparison, or that of any comparison with a p -value greater than this rejected comparison. This conclusion holds even when these p -values are smaller than their respective adjusted critical α value.

The exceptions to the application of this false-discovery rate algorithm are the post-hoc Tukey-Kramer tests performed for each significant ANOVA test, as these already take the statistical distributions associated with systematic repeated testing into account by making use of a Studentized range (Keuls, 1952). These tests are also considered conservative for comparisons of samples of unequal sizes like those considered here (Hayter, 1984; Somerville, 1993), and so are unlikely to result in type I errors.

3.3 Results

In total, we collected data from 25 individuals and recorded 7,054 wingbeats. The videos acquired clearly demonstrate that the *RS* and *PWP* do indeed come into contact during certain wingbeats (supplementary video 3.1), engaging in four types of interaction. Across all wingbeats, gear 3 was the most common (52.18 % of all wingbeats), followed by gear 1 (25.56 %) and then by gear 2 (18.95 %), with gear 0 wingbeats being by far the least common (3.22 %). A further 0.09 % of wingbeats were unclassifiable due to obfuscation of the hinge by the wing or legs. This variation was not uniform across recordings, as different individuals appear to display different propensities towards certain gears (figure 3.5). Given that the experimental conditions were identical across recordings, this may represent different individuals engaging in saccade behaviour to different extents (Schilstra and van Hateren, 1999; Dickinson, 2005; Bender and Dickinson, 2006; Hesselberg and Lehmann, 2007; Cheng et al., 2010). It is also interesting to note that similar variation across individuals has also been observed with alula flipping behaviour (Walker et al., 2012).

A gear 0 wingbeat was classified as the *RS* hooking behind the *PWP*. During this interaction, the two structures knock together (again with a similar storage and release of elastic energy), but the wingbeat is severely inhibited. These wingbeats were generally seen as the fly stopped beating its wings at the end of a bout of flight, or during incidences where the wings were vibrating and not producing a wingbeat.

During a gear 1 or 2 wingbeat, the wing moves antero-ventrally and near the end of the downstroke, or very shortly after it, the *RS* locks into the anterior or posterior groove of the *PWP* respectively ($t/T_{Down} = 0.8068 \pm 0.1$, and $t/T_{Down} = 0.9284 \pm 0.9$; mean $\pm$ one standard deviation, for gear 1 and gear 2 respectively; t is time and equal to zero at the start of the downstroke, and T_{Down} is the total length of the downstroke). The *RS* and *PWP* remain engaged shortly before the halfway point of the upstroke ($t/T_{Up} = 0.4884 \pm 0.1$, $t/T_{Up} = 0.3616 \pm 0.1$, for gear 1 and 2, respectively; T_{Up} is the total duration of the upstroke), at which point the *RS* quickly flicks out of the groove, with a distinct jerking motion seen around the base

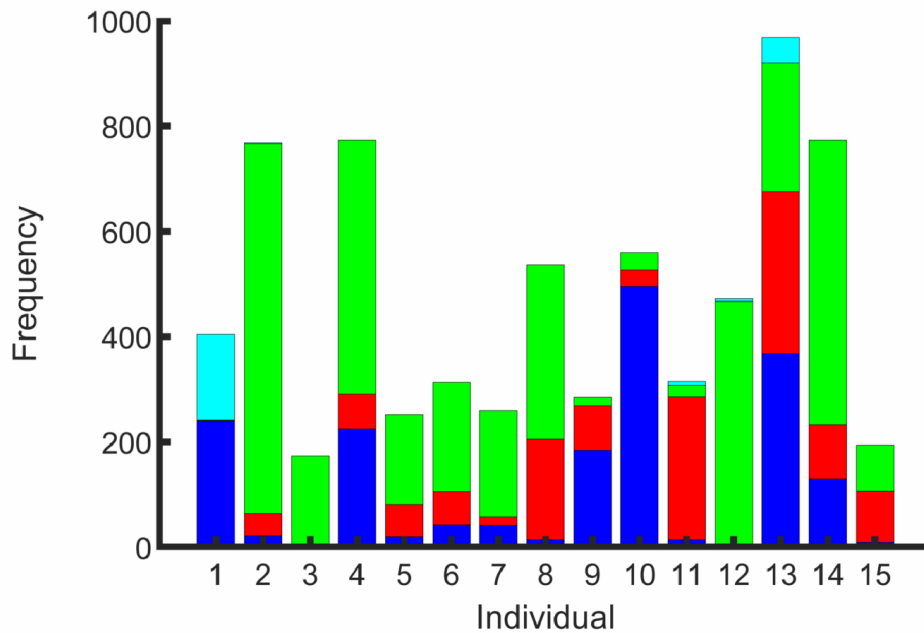


Figure 3.5: A bar graph showing the number of wingbeats in different gears by individual, broken down into different gears. Green - gear 3 ($n = 3,681$); Red - gear 2 ($n = 1,337$); Blue - gear 1 ($n = 1,803$); Cyan - gear 0 ($n = 227$); total $n = 7,048$ wingbeats from 15 individuals.

of the radial vein (examples of gear 1 and 2 wingbeats can be seen in supplementary videos 3.2 and 3.3, respectively; figure 3.6).

In contrast, a gear 3 wingbeat involved no contact between the *RS* and *PWP* (supplementary video 3.4). The wing is therefore free to move uninhibited during this type of wingbeat, and the *RS-PWP* interaction has no influence on wing kinematics.

While most of these interactions occurred on the downstroke, three recordings (amounting to 340 wingbeats) display the two structures interacting at the very start of the upstroke (see supplementary video 3.5 & figure 3.9). Both upstroke and downstroke interactions have been suggested before (e.g. Pfau (1987); Nalbach (1989)), but have previously been thought to be mutually exclusive theories. The videos, however, show that both of these types of interactions are possible.

These upstroke engagements, however, seem to be associated with extremely small stroke amplitudes wingbeats, which are unlikely to produce enough force to even support the body. These may therefore represent an extreme type of wing

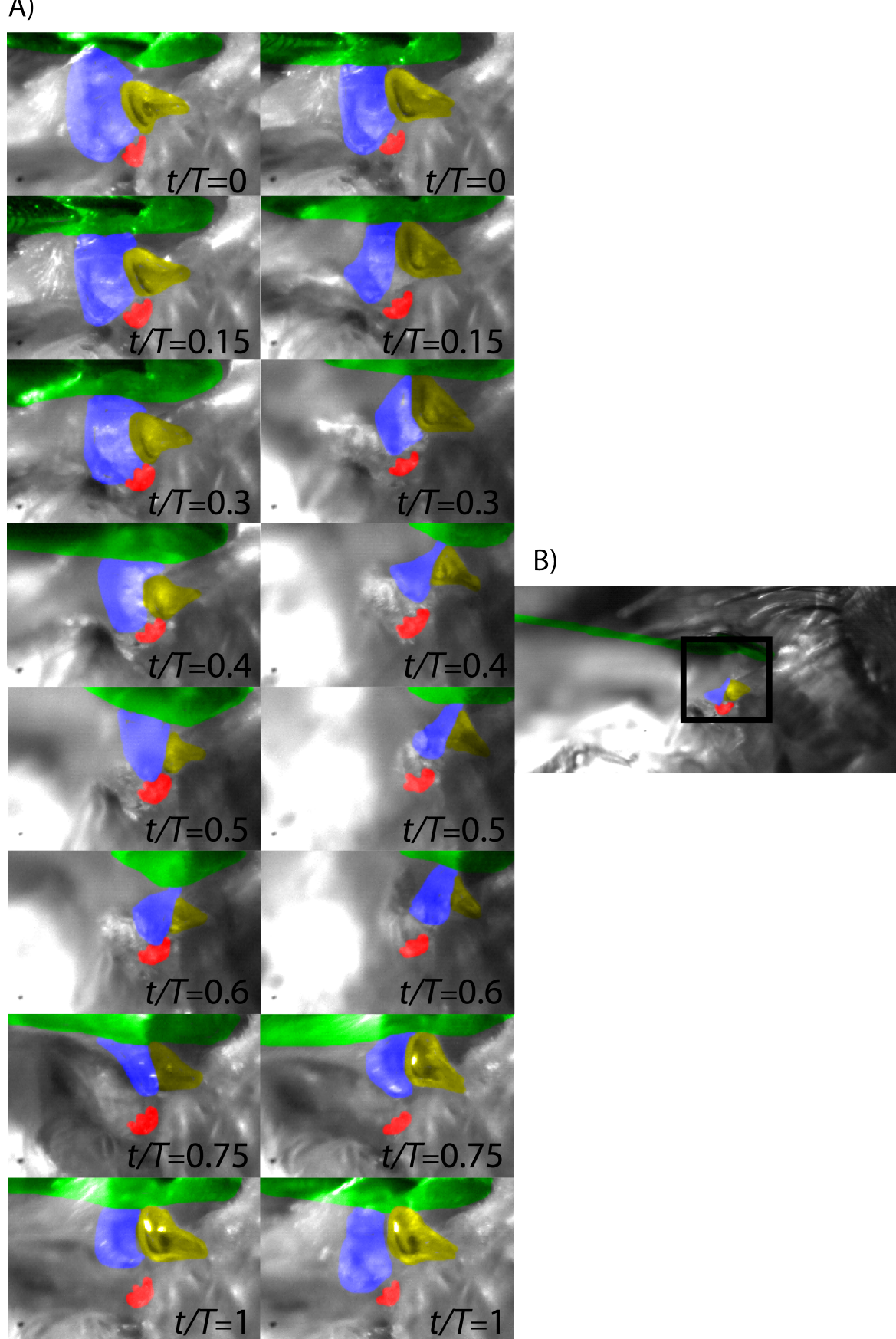


Figure 3.6: A) A series of zoomed-in frames adapted from videos focusing on the wing hinge during gear two (left) and a gear 3 (right) wingbeats. Anterior is to the right, the leading edge of the wing is in green, the *PtC* in yellow, the *RS* in blue, and the *PWP* in red. The relative time through the wingbeat is shown in the bottom right hand corner of each frame. T is equal to the total length of the wingbeat, while t is equal to time, where $t = 0$ at the start of the downstroke. These frames are adapted from the first wingbeats of supplementary videos 3.3 (gear 2) and 3.4 (gear 3). B) Zoomed-out picture of the wing hinge for reference.

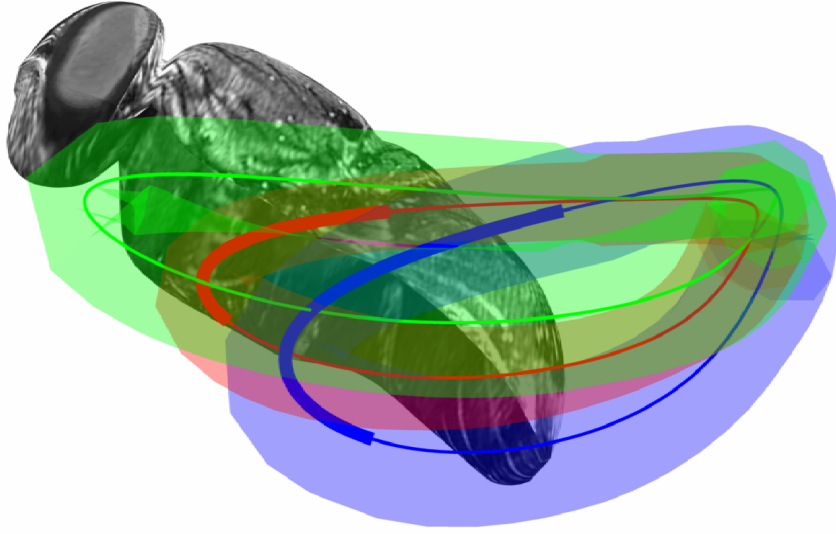


Figure 3.7: Three-Dimensional wingtip trajectories throughout the wingbeat in gears 1, 2 and 3. The thin, solid lines show the means of each gear, while the transparent surrounding areas show one standard deviation of these means. The thickened areas of the red and blue lines indicate the mean period over which the RS and PWP are engaged in gears 2 and 1, respectively. Green - gear 3 ($n = 3,681$); Red - gear 2 ($n = 1,337$); Blue - gear 1 ($n = 1,803$); total $n = 6,821$ wingbeats from 15 individuals.

movement that is sometimes encountered in tethered flight, just as wingbeats with only one flapping wing have been observed during previous tethered flight studies (Nachtigall and Wilson, 1967). Due to their unusual nature, though, and the fact that they were only seen in a very small number of recordings, these wingbeats have been omitted from further analysis.

3.3.1 Wing Kinematics in Different Gears

Having initially performing two-way ANOVA tests which included both the gear and individual as independent variables, we determined that, while wing average wing kinematics were often significantly different across individuals, the gear of a wingbeat, or the interaction between gear engaged and individual, were also significant factors despite this. From this, we conclude that the gear engaged during a wingbeat has a significant effect on the kinematics of the wing across individuals,

and that the relative differences in wing kinematics across wingbeats engaged in different gears will be seen regardless of the individual observed. For this reason, when reporting the number of observations (n) in this chapter and the remainder of this thesis, we report the number of wingbeats analysed rather than just the number of individuals.

We found significant differences in a number of the measured wing kinematics across wingbeats engaged in gears 1, 2 and 3 (figure 3.7). These kinematics include stroke angle, stroke deviation angle, stroke plane angle, wing pitch angle, and wingbeat frequency (table 3.1). Each of these kinematics play a substantial role in determining the force output of the flight motor, and in the control of whole-body manoeuvres.

Differences in stroke angle were amongst some of the most obvious discrepancies across the gears, and these will have a large effect on the magnitude of the force output from the wing. It is important to note that the positions of the wing at the end of the upstroke were far more similar amongst gears than are the positions at the end of the downstroke (figures 3.7 & 3.8). Maximum stroke angles (stroke angle at the end of the downstroke) are indeed different across gears ($F_{2,6818} = 2009$, $p < 0.001$), and Tukey-Kramer tests reveal that mean maximum stroke angle of wingbeats in gear 1 were significantly lower than those of gear 2 wingbeats ($q_{3,3140} = 28.53$, $p < 0.001$), which in turn were lower than the mean maximum stroke angle of wingbeats in gear 3 ($q_{3,5018} = 46.14$, $p < 0.001$). Minimum stroke angles also differ significantly across gears ($F_{2,6818} = 18.73$, $p < 0.001$), though there is less discrepancy between individual gears. Gear 2 wingbeats displayed the smallest minimum stroke angle on average, being significantly smaller than both gear 1 ($q_{3,3140} = 5.326$, $p < 0.001$) and gear 3 wingbeats ($q_{3,5018} = 8.648$, $p < 0.001$), but there was no significant difference between those of gears 1 and 3 ($q_{3,5484} = 2.919$, $p = 0.097$).

Since stroke amplitude is the difference between the stroke angles at the end of the upstroke and downstroke (which normally equal the minimum and maximum stroke angles, respectively), it is therefore no surprise that the differences we have

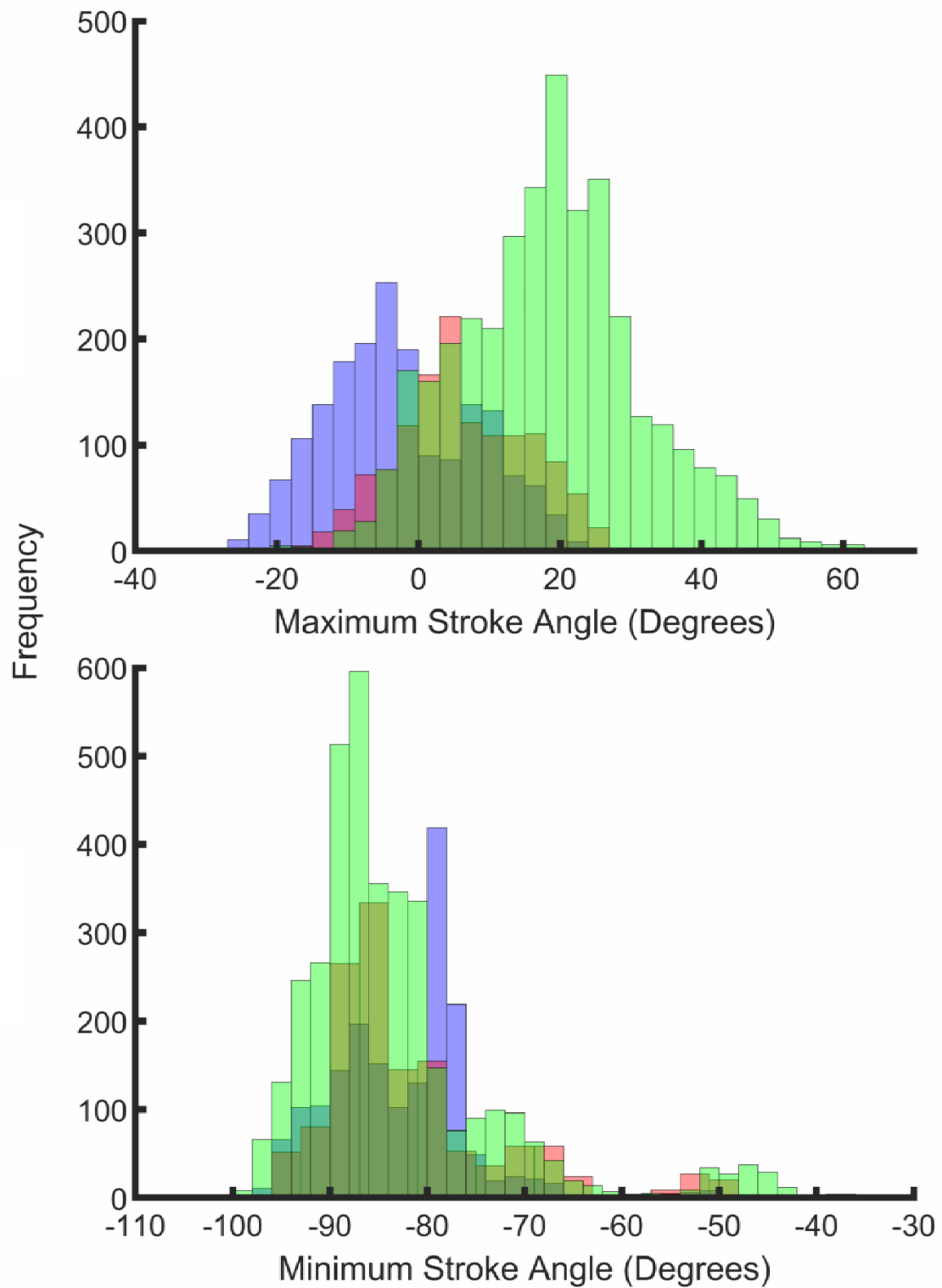


Figure 3.8: Histograms of the minimum (top) and maximum (bottom) stroke angles during wingbeats engaged in different gears. Green - gear 3 ($n = 3,681$); Red - gear 2 ($n = 1,337$); Blue - gear 1 ($n = 1,803$); total $n = 6,821$ wingbeats from 15 individuals.

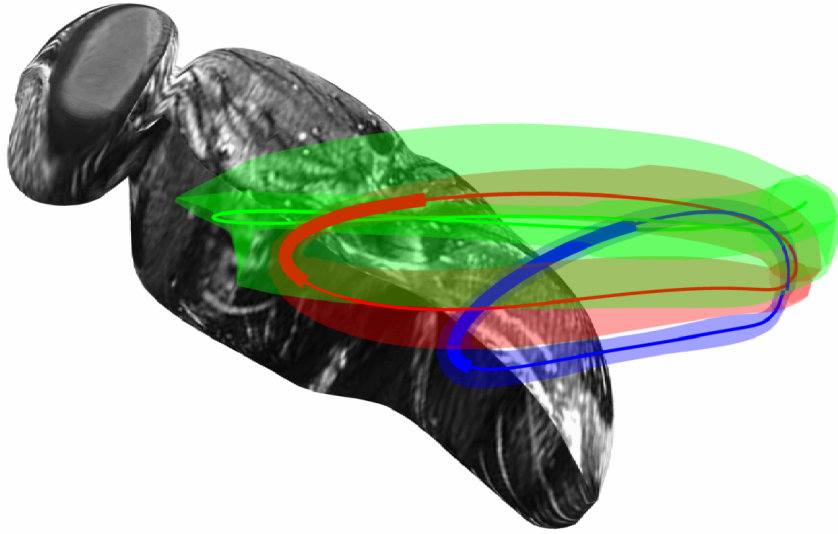


Figure 3.9: An example of wingtip trajectories during sequences in which the *RS* and *PWP* engaged during the upstroke. Blue – gear 1; Red – gear 2; Green – gear 3. The thin, solid lines show the means of each gear, while the transparent surrounding areas show one standard deviation of these means. The thickened areas of the red and blue lines indicate the mean period over which the *RS* and *PWP* are engaged in gears 2 and 1, respectively. Green - gear 3 ($n = 8$); Red - gear 2 ($n = 51$); Blue - gear 1 ($n = 8$); total $n = 68$ wingbeats from 1 individual.

found in maximum and minimum stroke angles across gears also lead to significant variation in stroke amplitudes across gears ($F_{2,6818} = 1187$, $p < 0.001$; table 3.1). Gear 1 displayed the lowest mean stroke amplitude, while gear 2 showed significantly greater mean stroke amplitude ($q_{3,3140} = 19.7$, $p < 0.001$) and mean gear 3 stroke amplitudes were greater still ($q_{3,5018} = 37.45$, $p < 0.001$).

Similarly, there were also significant differences in the range of stroke deviation angles exhibited between gears ($F_{2,6818} = 119.5$, $p < 0.001$; figure 3.11, table 3.1). Gear 1 showed the greatest range of stroke deviation angles across the wingbeat, larger than the range displayed by both gear 2 ($q_{3,3140} = 17.14$, $p < 0.001$) and gear 3 wingbeats ($q_{3,5484} = 20.64$, $p < 0.001$). However, there was no significant difference in stroke deviation range between gears 2 and 3 ($q_{3,5018} = 0.7918$, $p = 0.841$).

Despite this similarity, the source of the variation in stroke deviation varied between gears 2 and 3. Minimum stroke deviation angles differed significantly across gears ($F_{2,6818} = 151.9$, $p < 0.001$), and Tukey-Kramer tests revealed that gear 3

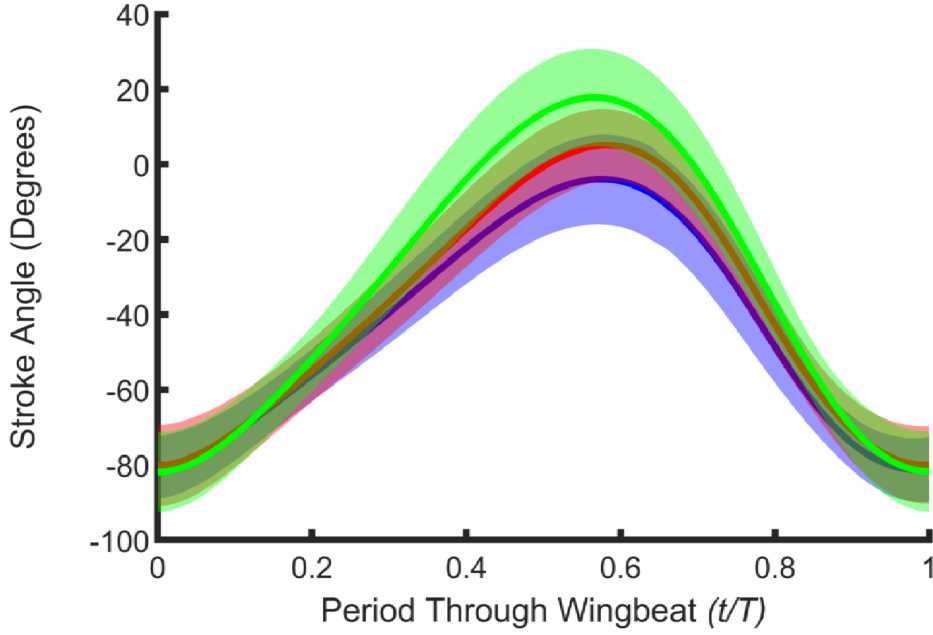


Figure 3.10: Stroke angle plotted over the course of wingbeats engaged in different gears. The thin bold line represents the mean, while the surrounding patch of the same colour shows one standard deviation of this mean. t is time, and T is the total duration of the wingbeat, where t is equal to zero at the start of the downstroke. Green - gear 3 ($n = 3,681$); Red - gear 2 ($n = 1,337$); Blue - gear 1 ($n = 1,803$); total $n = 6,821$ wingbeats from 15 individuals.

exhibited the greatest, being significantly larger than both the minimum stroke deviation angles of gear 2 ($q_{3,5018} = 21.38$, $p < 0.001$) and gear 1 wingbeats ($q_{3,5484} = 18.04$, $p < 0.001$). Minimum stroke deviation angle also differed between gears 1 and 2, with gear 2 displaying the smallest values ($q_{3,3140} = 4.554$, $p = 0.004$). Maximum stroke deviation showed similar discrepancies across gears ($F_{2,6818} = 619.8$, $p < 0.001$), but gear 1 wingbeats showed the largest, being greater than those of both gear 2 ($q_{3,3140} = 22.49$, $p < 0.001$) and gear 3 wingbeats ($q_{3,5484} = 49.57$, $p < 0.001$). Gear 3 showed the smallest maximum deviation angle, also being significantly smaller than gear 2 ($q_{3,5018} = 19.2$, $p < 0.001$). These results suggest that gear 1 wingbeats possess the greatest deviations from the stroke plane over the course of the wingbeat compared to gears 2 or 3, in which the wingtip is, on average, closer to it. Furthermore, the deviations from the stroke plane differed between gears 2 and 3, in that gear 3 deviates further ventrally to the stroke plane, while gear 2 wingbeats deviate more dorsally to it.

The plane around which this deviation occurs also changed across gears ($F_{2,6818} = 80.61$, $p < 0.001$; table 3.1). There is a small but significant difference between the stroke planes of gears 1 and 2 ($q_{3,3140} = 3.608$, $p = 0.029$), while gear 3 wingbeats showed a significantly more horizontal stroke plane compared to both gears 1 ($q_{3,5484} = 12.94$, $p < 0.001$) and 2 ($q_{3,5018} = 15.72$, $p < 0.001$; figure 3.7).

Table 3.1: Kinematics of the wings during different gears, presented as the mean $\pm$ one standard deviation and the resultant F - and p -values resulting from a one-way ANOVA test. Bold values indicate significant results after the application of a false discovery rate algorithm. φ = stroke angle (φ_{Range} therefore represents stroke amplitude), ϑ = deviation angle, α = wing pitch angle, ψ = stroke plane angle.

Kinematic	Gear 1	Gear 2	Gear 3	Significance
φ_{Max}	-2.683 $\pm$ 11°	6.076 $\pm$ 9°	18.61 $\pm$ 13°	$F_{2,6818} = 2009$, $p < 0.001$
φ_{Min}	-82.68 $\pm$ 7°	-81.41 $\pm$ 10°	-83.24 $\pm$ 10°	$F_{2,6818} = 18.73$, $p < 0.001$
φ_{Range}	77.9 $\pm$ 14°	86.32 $\pm$ 16°	100.5 $\pm$ 18°	$F_{2,6818} = 1187$, $p < 0.001$
ϑ_{Max}	15.71 $\pm$ 7°	11.63 $\pm$ 7°	8.552 $\pm$ 7°	$F_{2,6818} = 619.8$, $p < 0.001$
ϑ_{Min}	-11.21 $\pm$ 5°	-10.42 $\pm$ 5°	-13.7 $\pm$ 8°	$F_{2,6818} = 151.9$, $p < 0.001$
ϑ_{Mean}	0.1803 $\pm$ 2°	-0.5559 $\pm$ 1°	-2.488 $\pm$ 4°	$F_{2,6818} = 449.6$, $p < 0.001$
ϑ_{Range}	26.92 $\pm$ 12°	22.05 $\pm$ 11°	22.25 $\pm$ 11°	$F_{2,6818} = 119.5$, $p < 0.001$
ψ_{Mean}	-7.323 $\pm$ 6°	-7.849 $\pm$ 5°	-5.82 $\pm$ 6°	$F_{2,6818} = 80.61$, $p < 0.001$
Mid-Upstroke α	127.1 $\pm$ 27°	129.2 $\pm$ 27°	145 $\pm$ 22°	$F_{2,5911} = 353.5$, $p < 0.001$
Mid-Downstroke α	25.48 $\pm$ 20°	25.01 $\pm$ 13°	26.31 $\pm$ 13°	$F_{2,5911} = 3.596$, $p = 0.026$
Relative Timing of Supination (t/T)	0.6507 $\pm$ 0.09	0.6623 $\pm$ 0.1	0.6491 $\pm$ 0.07	$F_{2,6030} = 10.74$, $p < 0.001$
Relative Timing of Downstroke End (t/T)	0.5726 $\pm$ 0.04	0.5747 $\pm$ 0.04	0.5626 $\pm$ 0.03	$F_{2,6818} = 78.98$, $p < 0.001$
Wingbeat Frequency (Hz)	167.7 $\pm$ 19	157.2 $\pm$ 19	156.9 $\pm$ 15	$F_{2,6818} = 268$, $p < 0.001$

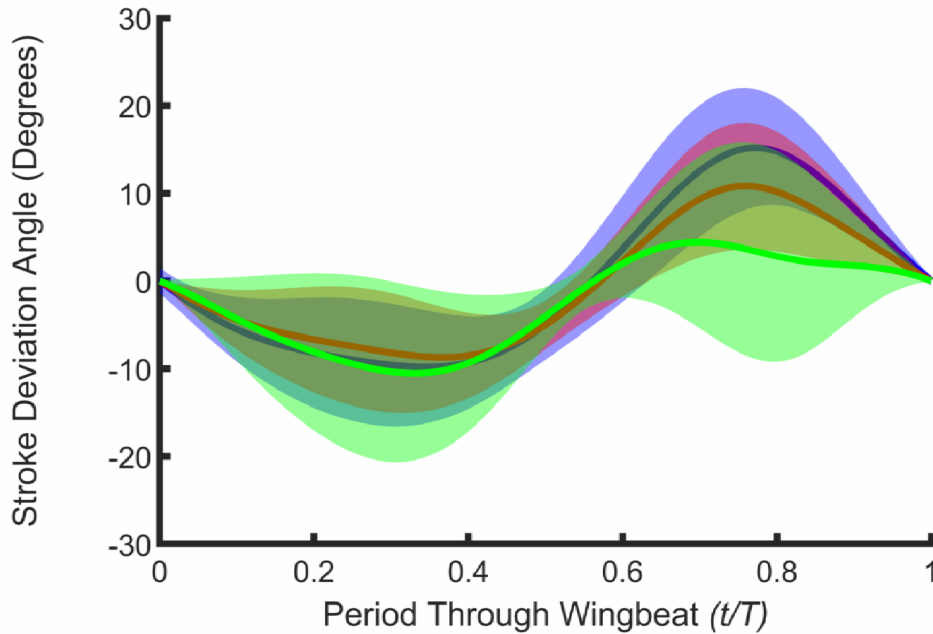


Figure 3.11: Stroke deviation angle plotted over the course of wingbeats engaged in different gears. The thin bold line represents the mean, while the surrounding patch of the same colour shows one standard deviation of this mean. Green - gear 3 ($n = 3,681$); Red - gear 2 ($n = 1,337$); Blue - gear 1 ($n = 1,803$); total $n = 6,821$ wingbeats from 15 individuals.

Gear changes also seem to be correlated with variations in wing kinematics that are not associated with the wing's trajectory. Wingbeat frequency, for example, varied across gears ($F_{2,6818} = 268$, $p < 0.001$; table 3.1) with gear 1 showing significantly greater frequencies than both gear 2 ($q_{3,3140} = 24.35$, $p < 0.001$) and gear 3 wingbeats ($q_{3,5484} = 31.52$, $p < 0.001$), while gears 2 and 3 were more similar ($q_{3,5018} = 0.848$, $p = 0.82$).

Finally, the measured wing pitch angles at the mid-upstroke also exhibited significant variations across the three gears ($F_{2,5911} = 353.5$, $p < 0.001$). Gear 3 wingbeats showed mid-upstroke wing pitch angles that were greater than those of both gear 1 ($q_{3,4765} = 33.87$, $p < 0.001$) and gear 2 ($q_{3,4193} = 26.15$, $p < 0.001$), while gear 1 and 2 wingbeats displayed no significant differences between their mean mid-upstroke wing pitch angles ($q_{3,2870} = -3.045$, $p = 0.08$). The wing pitch angle measured at the mid-downstroke, however, was the only wing kinematic measured here not to show significant variation across the three gears ($F_{2,5911} = 3.596$, $p < 0.001$; figure 3.12,

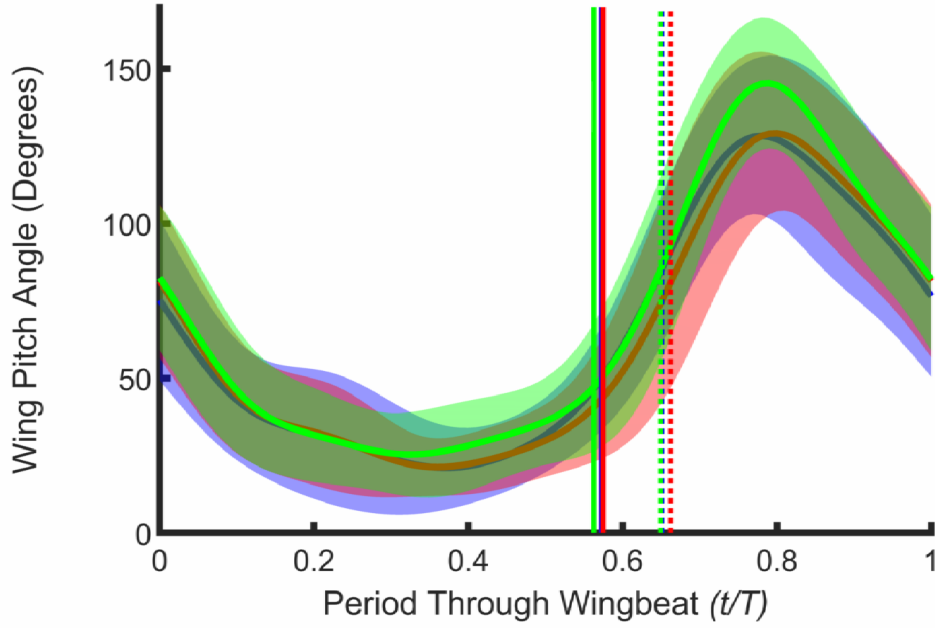


Figure 3.12: Wing pitch angle plotted over the course of wingbeats engaged in different gears. The thin bold line represents the mean, while the surrounding patch of the same colour shows one standard deviation of this mean. The solid vertical lines represent the mean timing of the end of the downstroke, and the dotted vertical line shows the mean timing of wing supination. Note that the blue vertical lines representing the timings for gear 1 are slightly obscured by the red lines representing gear 2 timings. Green - gear 3 ($n = 3,044$); Red - gear 2 ($n = 1,149$); Blue - gear 1 ($n = 1,721$); total $n = 5,914$ wingbeats from 15 individuals.

table 3.1).

There are further kinematics that can be extracted from the wing pitch angle, such as the proportion of the wingbeat taken up by the downstroke, and the timing of wing supination (measured as the point at which the wing rotates to a pitch angle of 90° near the end of the downstroke). The length of the downstroke was significantly different depending on whether the *RS* and *PWP* made contact during the wingbeat ($F_{2,6818} = 78.98$, $p < 0.001$; table 3.1), with gear 3 wingbeats exhibiting shorter downstrokes compared to both gear 2 ($q_{3,5018} = 14.92$, $p < 0.001$) and gear 1 wingbeats ($q_{3,5484} = 13.64$, $p < 0.001$), while the length of the downstroke did not change significantly between gears 1 and 2 ($q_{3,3140} = 2.337$, $p = 0.224$). The timing of wing supination also varied across wingbeats of different gears ($F_{2,6030} = 1074$, $p < 0.001$). Gear 2 wingbeats showed the most delayed wing supination of the three gears ($q_{3,2946} = 5.115$, $p < 0.001$; $q_{3,4304} = 6.439$, $p < 0.001$; compared against the

mean supination timings of gears 1 and 3 respectively), while gears 1 and 3 occurred earlier, but did not differ significantly between each other ($q_{3,4816} = 0.8842$, $p = 0.806$).

3.3.2 The Alula as an Indicator of the Gear Change Mechanism

As well as variation in the gears utilised by the insects, there was also a great deal of variation in the states of the alula between different individuals (figure 3.13). A flipped alula was the most common state (73.38% of 7,054 wingbeats), while flat alula wingbeats were far less common (26.62%).

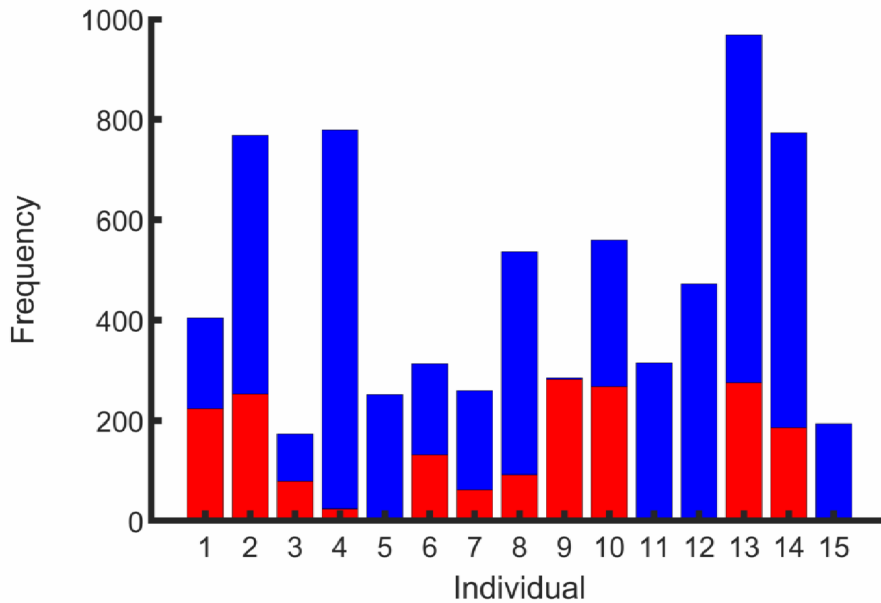


Figure 3.13: A bar graph showing the number of wingbeats per individual recorded, broken down into different alula states. Blue - flipped alula ($n = 1,878$); red - flat alula ($n = 5,176$); total $n = 7,054$ wingbeats from 15 individuals.

A χ^2 test reveals that there was a significant relationship between the gear of the wingbeat, and the state of the alula ($\chi^2 = 548.4$, $p < 0.001$). However, the alula cannot be used as a definitive indicator of the gear change mechanism, as it can occupy a flipped or a flat state regardless of which gear is engaged (table 3.2). Post-hoc tests also show that, despite there not being a definitive relationship, the

Table 3.2: The state of the alula during differently geared wingbeats. $n = 6,821$ wingbeats. $\chi^2 = 548.4$, $p < 0.001$.

	Gear State			
Alula State	<i>Gear 1</i>	<i>Gear 2</i>	<i>Gear 3</i>	Total
<i>Flat</i>	874	224	780	1878
<i>Flipped</i>	929	1113	2901	4943
Total	1803	1337	3681	6821

Table 3.3: The adjusted residual values for each gear-alula state combination. Bold entries indicate residuals with a significantly large residual, indicating significantly fewer (negative residual) or greater (positive residual) instances of this gear-alula state combination than predicted by the null hypothesis. Critical value = 2.65 after applying a Bonferroni correction.

	Gear State		
Alula State	<i>Gear 1</i>	<i>Gear 2</i>	<i>Gear 3</i>
<i>Flat</i>	23.21	-9.84	-12.7
<i>Flipped</i>	-23.21	9.84	12.7

Table 3.4: Results of ransacking performed on the data within table 3.2. z -values in bold indicate significant differences (greater than the critical z -value of 2.45) between the alula state pattern recorded among the wingbeat gears being compared.

Gear Comparison z-Values					
Gear 1 Gear 2		Gear 1 Gear 3		Gear 2 Gear 3	
16.07		13.05		3.0182	

alula may be useful as an indicator of the probability of which gear is engaged.

Using a significance value of 95% adjusted with a Bonferroni correction, we find that gear 1 wingbeats were more likely to have a flat rather than a flipped alula, while gear 2 and 3 wingbeats were proportionately more likely to have the alula in its flipped state. Furthermore, ransacking the data within table 3.2 shows that the alula state patterns of gear 1 wingbeats were significantly different to those of gears 2 and 3, but there was no significant difference between the alula state patterns of wingbeats in gear 2 and 3. Together, these results suggest that wingbeats showing a flat alula are most likely to be in gear 1, while alula-flipped wingbeats are more likely to be engaged in gears 2 or 3, though it is difficult to distinguish between the latter two.

3.3.3 Alula State Changes and Wingbeat Kinematics

Just as wingbeat kinematics vary depending on the gear engaged, they also appear to change depending on the state of the alula. When examining the stroke angle throughout the wingbeat in different alula states, it becomes clear that alula-flat wingbeats showed a significantly greater maximum stroke angle compared to those with a flipped alula ($t_{7052} = 5.469$, $p < 0.001$; table 3.5, figure 3.14). Furthermore, alula-flat wingbeats reached a greater minimum stroke angle too when compared with alula-flipped wingbeats ($t_{7052} = 22.31$, $p < 0.001$). This accounts for the significant difference in stroke amplitude between the two alula states, in which alula-flat wingbeats beat with the greatest stroke amplitude of the two ($t_{7052} = 15.13$, $p < 0.001$).

Similarly, the stroke deviation angle of the wingbeat, while not as clearly different as when comparing across gears, varied significantly between the alula states (figure 3.15, table 3.5). The total range of deviation of alula-flipped wingbeats were slightly greater than alula-flat wingbeats ($t_{7052} = 6.7$, $p < 0.001$). This greater range during alula-flat wingbeats stems from its greater minimum deviation angle ($t_{7052} = 7.32$, $p < 0.001$), and maximum deviation angle when compared to alula-flipped wingbeats

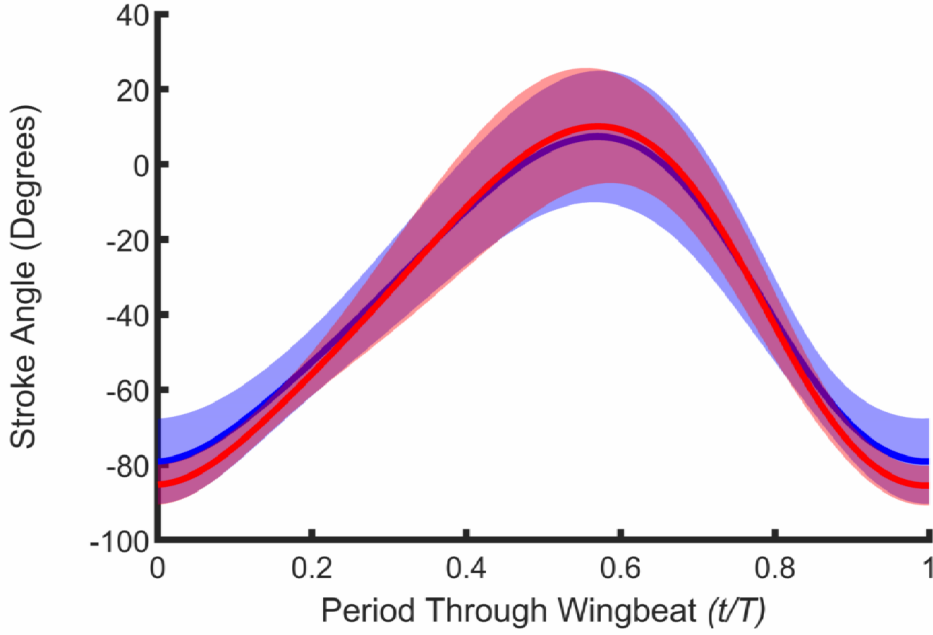


Figure 3.14: Stroke angle plotted over the course of wingbeats exhibiting different alula states. The thin bold line represents the mean, while the surrounding patch of the same colour shows one standard deviation of this mean. Blue - flipped alula ($n = 1,878$); red - flat alula ($n = 5,176$); total $n = 7,054$ wingbeats from 15 individuals.

($t_{7052} = 3.297$, $p < 0.001$). The position of the stroke plane around which this deviation occurs was also significantly more horizontal in flat-alula wingbeats when compared to those in which the alula was flipped ($t_{7052} = 9.419$, $p < 0.001$).

When the mid-stroke wing pitch angles throughout the wingbeat are examined, similar differences to those found when comparing different gears can be seen (figure 3.16, table 3.5). Alula-flat wingbeats exhibited significantly greater mid-stroke wing pitch angles during both the downstroke ($t_{6143} = 5.381$, $p < 0.001$) and the upstroke ($t_{6143} = 14.44$, $p < 0.001$), just as gear 3 showed greater values compared to gear 1 and 2. Similarly, alula-flat wingbeats supinated significantly earlier in the wingbeat compared to flipped-alula wingbeats ($t_{6079} = 8.558$, $p < 0.001$). However, this earlier supination is not accompanied by a proportionately shorter downstroke, as alula-flat wingbeats show a downstroke which ends significantly later end of the downstroke when compared to alula-flipped wingbeats ($t_{7052} = 8.881$, $p < 0.001$).

Finally, we also found significant differences in the wingbeat frequencies of alula states ($t_{7052} = 8.754$, $p < 0.001$), with alula-flat wingbeats showing higher mean

values than alula-flipped wingbeats.

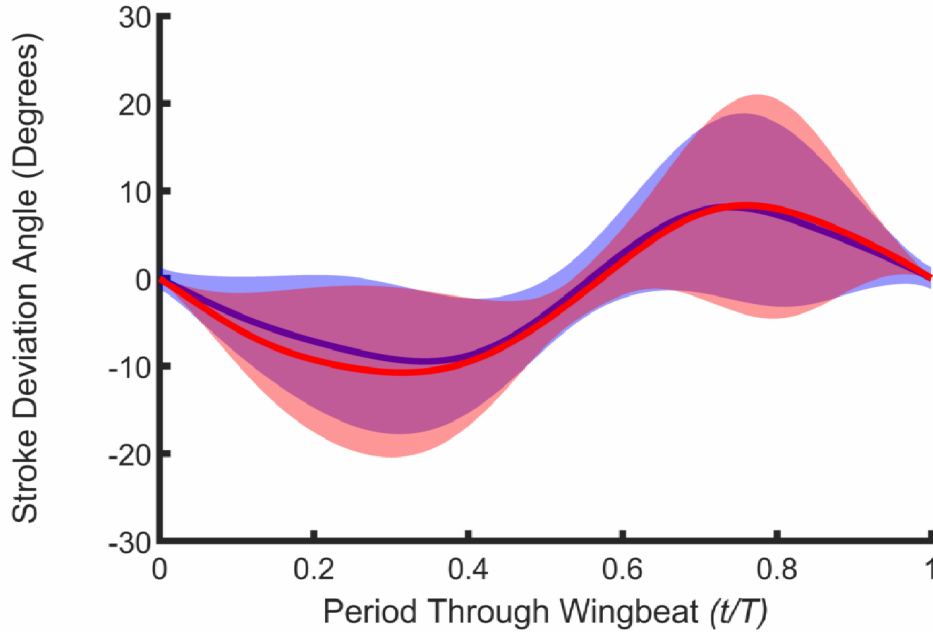


Figure 3.15: Stroke deviation angle plotted over the course of wingbeats exhibiting different alula states. The thin bold line represents the mean, while the surrounding patch of the same colour shows one standard deviation of this mean. Blue - flipped alula ($n = 1,878$); red - flat alula ($n = 5,176$); total $n = 7,054$ wingbeats from 15 individuals.

The significant differences between alula states are maintained as we inspect the different alula states within each gear (table 3.6 and figure 3.17). Two-way t-tests reveal that, while wingbeats do not differ significantly across each measured kinematic and within each gear, there are a large number of significant discrepancies, particularly maximum stroke angle and stroke amplitude, in which flat-alula wingbeats consistently exhibit greater values. This suggests that whichever muscular mechanism is responsible for bringing the alula into a flat state may also be involved in causing the wingtip to travel further anteriorly during the downstroke. This combination of greater stroke amplitude and greater wing area due to the flattened alula further implies that wingbeats such as these are adapted to accelerate the largest amount of air and produce the greatest amount of aerodynamic force as a result. This effect will be further enhanced by the higher wingbeat frequencies that also occur during flat-alula wingbeats.

Table 3.5: Kinematics of the wings during different alula states, presented as the mean $\pm$ one standard deviation and the resultant t - and p -values resulting from a two sample t-test. Bold values indicate significant results after the application of a false discovery rate algorithm. φ = stroke angle (φ_{Range} therefore represents stroke amplitude), ϑ = deviation angle, α = wing pitch angle, ψ = stroke plane angle.

Kinematic	Flat Alula	Flipped Alula	Significance
φ_{Max}	$10.99 \pm 15^\circ$	$8.542 \pm 17^\circ$	$t(7052) = 5.469$, $p < 0.001$
φ_{Min}	$-86.41 \pm 5^\circ$	$-80.77 \pm 10^\circ$	$t(7052) = 22.31$, $p < 0.001$
φ_{Range}	$96.26 \pm 16^\circ$	$87.52 \pm 23^\circ$	$t(7052) = 15.13$, $p < 0.001$
ϑ_{Max}	$11.53 \pm 7^\circ$	$10.83 \pm 8^\circ$	$t(7052) = 3.297$, $p < 0.001$
ϑ_{Min}	$13.22 \pm 8^\circ$	$11.85 \pm 7^\circ$	$t(7052) = 7.32$, $p < 0.001$
ϑ_{Mean}	$-1.788 \pm 5^\circ$	$-1.147 \pm 3^\circ$	$t(7052) = 6.677$, $p < 0.001$
ϑ_{Range}	$24.74 \pm 10^\circ$	$22.68 \pm 12^\circ$	$t(7052) = 6.7$, $p < 0.001$
ψ_{Mean}	$-5.249 \pm 4^\circ$	$-6.839 \pm 7^\circ$	$t(7052) = 9.419$, $p = 0.001$
Mid-Upstroke α	$143.1 \pm 25^\circ$	$130.8 \pm 31^\circ$	$t(6143) = 14.44$, $p < 0.001$
Mid-Downstroke α	$27.74 \pm 9^\circ$	$25.14 \pm 19^\circ$	$t(6143) = 5.381$, $p < 0.001$
Relative Timing of Supination (t/T)	0.6356 ± 0.06	0.6571 ± 0.09	$t(6079) = 8.558$, $p < 0.001$
Relative Timing of Downstroke End (t/T)	0.5722 ± 0.03	0.5631 ± 0.04	$t(7052) = 8.881$, $p < 0.001$
Wingbeat Frequency (Hz)	164.2 ± 17	159.8 ± 19	$t(7052) = 8.754$, $p < 0.001$

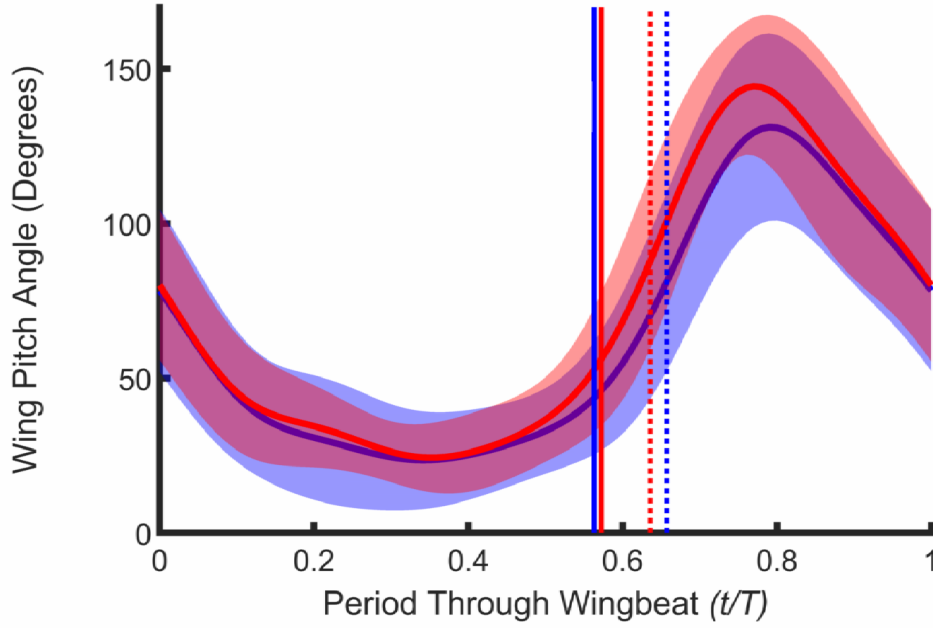


Figure 3.16: Wing pitch angle plotted over the course of wingbeats exhibiting different alula states. The thin bold line represents the mean, while the surrounding patch of the same colour shows one standard deviation of this mean. The solid vertical lines represent the mean timing of the end of the downstroke, and the dotted vertical line shows the mean timing of wing supination. Blue - flipped alula ($n = 1,601$); red - flat alula ($n = 4,544$); total $n = 6,145$ wingbeats from 15 individuals.

Table 3.6: Comparisons of wing kinematics between different alula states within each gear, presented as the mean $\pm$ one standard deviation, and the resultant t - and p - values resulting from a two-sample t -test. Bold values indicate significant results after the application of a false discovery rate algorithm. φ = stroke angle (φ_{Range} therefore represents stroke amplitude), ϑ = deviation angle, α = wing pitch angle, ψ = stroke plane angle, t = time and T = the duration of the whole wingbeat, with t being equal to 0 at the start of the downstroke.

Kinematic	Gear	Alula State		Significance
		Flat	Flipped	
φ_{Max}	1	$1.478 \pm 10^\circ$	$-6.598 \pm 11^\circ$	$t(1801) = 16.02$, $p < 0.001$
	2	$11.34 \pm 12^\circ$	$5.016 \pm 9^\circ$	$t(1335) = 9.388$, $p < 0.001$
	3	$21.55 \pm 14^\circ$	$17.82 \pm 13^\circ$	$t(3679) = 7.116$, $p < 0.001$
φ_{Min}	1	$-85.92 \pm 6^\circ$	$-79.64 \pm 7^\circ$	$t(1801) = -20.65$, $p < 0.001$
	2	$-86.61 \pm 3^\circ$	$-80.36 \pm 11^\circ$	$t(1335) = -8.735$, $p < 0.001$
	3	$-86.9 \pm 5^\circ$	$-82.26 \pm 11^\circ$	$t(3679) = -11.6$, $p < 0.001$

φ_{Range}	1	$86.27 \pm 9^\circ$	$70.03 \pm 14^\circ$	$t(1801) = 29.41,$ $p < 0.001$
	2	$97.49 \pm 12^\circ$	$84.07 \pm 16^\circ$	$t(1335) = 12.14,$ $p < 0.001$
	3	$107.1 \pm 16^\circ$	$98.7 \pm 18^\circ$	$t(3679) = 11.73,$ $p < 0.001$
ϑ_{Max}	1	$14.22 \pm 6^\circ$	$17.11 \pm 9^\circ$	$t(1801) = -8.459,$ $p < 0.001$
	2	$11.77 \pm 4^\circ$	$11.6 \pm 7^\circ$	$t(1335) = 0.364,$ $p = 0.716$
	3	$8.447 \pm 8^\circ$	$8.58 \pm 7^\circ$	$t(3679) = -0.4625,$ $p = 0.644$
ϑ_{Min}	1	$-12.13 \pm 6^\circ$	$-10.24 \pm 5^\circ$	$t(1801) = 7.478,$ $p < 0.001$
	2	$-12.07 \pm 5^\circ$	$-10.09 \pm 5^\circ$	$t(1335) = -5.195,$ $p < 0.001$
	3	$-16.88 \pm 10^\circ$	$-12.85 \pm 7^\circ$	$t(3679) = -13.1,$ $p < 0.001$
ϑ_{Mean}	1	$-0.4132 \pm 11^\circ$	$0.1721 \pm 11^\circ$	$t(1801) = -1.135,$ $p = 0.256$
	2	$-0.3409 \pm 12^\circ$	$-0.1543 \pm 9^\circ$	$t(1335) = -0.2649,$ $p = 0.791$
	3	$-0.7426 \pm 10^\circ$	$-1.042 \pm 10^\circ$	$t(3679) = 0.71,$ $p = 0.478$
ϑ_{Range}	1	$24.46 \pm 10^\circ$	$29.23 \pm 13^\circ$	$t(1801) = -8.678,$ $p < 0.001$
	2	$23.84 \pm 8^\circ$	$21.69 \pm 11^\circ$	$t(1335) = 2.716,$ $p = 0.007$
	3	$25.33 \pm 9^\circ$	$21.43 \pm 11^\circ$	$t(3679) = 9.04,$ $p < 0.001$
ψ_{Mean}	1	$-5.629 \pm 4^\circ$	$-8.916 \pm 8^\circ$	$t(1801) = 11.16,$ $p < 0.001$
	2	$-7.562 \pm 4^\circ$	$-7.906 \pm 5^\circ$	$t(1335) = 0.9184,$ $p = 0.359$
	3	$-4.158 \pm 4^\circ$	$-6.267 \pm 6^\circ$	$t(3679) = 9.581,$ $p < 0.001$
Mid- Upstroke α	1	$137.6 \pm 25^\circ$	$116.3 \pm 25^\circ$	$t(1719) = 17.71,$ $p < 0.001$
	2	$144.1 \pm 28^\circ$	$125.6 \pm 26^\circ$	$t(1147) = 9.361,$ $p < 0.001$
	3	$152 \pm 21^\circ$	$143.6 \pm 22^\circ$	$t(3042) = 7.878,$ $p < 0.001$
Mid- Downstroke α	1	$26.27 \pm 9^\circ$	$24.66 \pm 27^\circ$	$t(1719) = 1.679,$ $p = 0.093$
	2	$30.29 \pm 7^\circ$	$23.74 \pm 14^\circ$	$t(1147) = 6.714,$ $p < 0.001$

	3	$29.16 \pm 10^\circ$	$25.75 \pm 13^\circ$	$t(3042) = 5.489,$ $p < 0.001$
Relative Timing of Supination (t/T)	1	0.6496 ± 0.04	0.6519 ± 0.1	$t(1727) = -0.5514,$ $p = 0.581$
	2	0.6289 ± 0.06	0.6698 ± 0.1	$t(1215) = -5.011,$ $p < 0.001$
	3	0.6156 ± 0.08	0.656 ± 0.07	$t(3085) = -11.98,$ $p < 0.001$
Relative Timing of Downstroke End (t/T)	1	0.5841 ± 0.03	0.5618 ± 0.05	$t(1801) = 11.57,$ $p < 0.001$
	2	0.5728 ± 0.03	0.5751 ± 0.04	$t(1335) = -0.7888,$ $p = 0.43$
	3	0.5586 ± 0.6	0.5637 ± 0.03	$t(3679) = -4.017,$ $p < 0.001$
Wingbeat Frequency (Hz)	1	166.8 ± 16	168.6 ± 21	$t(1801) = -2.11,$ $p = 0.035$
	2	170.6 ± 19	154.5 ± 17	$t(1335) = 12.52,$ $p < 0.001$
	3	159.6 ± 16	156.1 ± 15	$t(3679) = 5.604,$ $p < 0.001$

3.4 Discussion

The data presented here show that each gear is associated with significantly different sets of wing kinematics, which in turn will cause the wing to produce aerodynamic forces that differ both in magnitude and in direction. There are, for example, distinctive variations between each gear in terms of stroke amplitude, with wingbeats in gear 3 displaying the greatest stroke amplitude, while wingbeats in gear 1 show the lowest. This is in keeping with theories regarding the gear change mechanism, which suggest that *RS-PWP* interaction should inhibit wing movement during the downstroke as the interaction occurs (Pfau, 1973; Nalbach, 1989; Walker et al., 2012). Further supporting this idea is the fact that kinematics towards the start of the downstroke and the end of the upstroke (periods during which there is no *RS-PWP* contact), such as minimum stroke angle, show much less variation between gears than do kinematics during the second half of the downstroke and the first half of the upstroke (which is when *RS-PWP* contact occurs; figures 3.7 & 3.8).

If *RS-PWP* interaction does indeed act like a brake to restrict wing motion during the end of the downstroke this gives a mechanistic explanation for the variation in anterior movement of the wing during the wingbeat at this time, and explains the variation in maximum stroke angle across gears. Furthermore, the contact between the *RS* and *PWP* has the potential to alter the resonant frequency of the wing-thorax system, a feature which the dipteran flight motor is thought to rely on to maintain efficiency (Pringle, 1949; Roeder, 1951; Greenewalt, 1960; Dickinson and Lighton, 1995; Brennan et al., 2003; Ha et al., 2013; Deora et al., 2017). If the wing and thorax can be roughly modelled as an undamped rotating mechanical system (with the wing rotating around the thorax about the wing hinge), the resonant frequency (f_n) can be estimated as:

$$f_n = \frac{1}{2\pi} \sqrt{\frac{\mu}{I}}$$

Where (μ) is the torsion constant, and (I) is the moment of inertia of the system. I itself is defined as $I = mr^2$, in which m is the mass of the system, and r is the distance of the centre of mass from the principal axis of rotation (in this case this refers to the distance of the centre of mass of the wing from the wing hinge).

As the *RS* and *PWP* make contact, this alters the pivot point about which the wing rotates around the thorax. By effectively moving the axis of rotation proximally along the wing (i.e. reducing the effective length of the wing), the distance between the wing's centre of mass from the axis of rotation (r) is reduced. This thereby alters the moment of inertia experienced by the flight motor during flapping, which in turn influences the system's resonant frequency. If this is indeed the case, this explains the increase in mean wingbeat frequency observed across gears, with gear 3 wingbeats, where r is largest, showing the lowest wingbeat frequencies of all gears, and gear 1 wingbeats, where r is smallest, exhibiting the greatest wingbeat frequencies.

The pivot point formed by the *RS-PWP* is also likely to change the point about which the wing pitches at the end of the downstroke. Indeed, we find that the wing supinates later in the wingbeat when the *RS* and *PWP* come into contact (figure

3.12), corroborating findings by previous authors (Wisser, 1987; Ennos, 1987). Despite these observations of the mechanism and its implications for wing kinematics, we have not been able to determine how these different *RS-PWP* interactions are controlled. The muscular activity underlying the gear change mechanism, and the precise movement of the components involved, should therefore be a subject explored in future work.

Table 3.7: A table documenting the significance values of kinematic differences found associated with gear changing and alula state changing. Arrows indicate the trend of the kinematic value from gear 3 to gear 1, and from a flat to a flipped alula. $\uparrow$ and $\downarrow$ denotes an increasing or decreasing value, respectively, while $\rightarrow$ and $\leftarrow$ denotes the end of the downstroke or timing of supination occurring later or earlier in the wingbeat, respectively. Bold values indicate results which are statistically significant after the application of a false discovery rate algorithm. φ = stroke angle (φ_{Range} therefore represents stroke amplitude), ϑ = deviation angle, α = wing pitch angle, ψ = stroke plane angle.

Kinematic	Gear Change	Alula State Changes
φ_{Max}	$\downarrow - p < 0.001$	$\downarrow - p < 0.001$
φ_{Min}	$\downarrow - p < 0.001$	$\downarrow - p < 0.001$
	$\uparrow$ (gear 2 to gear 1)	
φ_{Range}	$\downarrow$ (gear 3 to 1) $p < 0.001$	$\downarrow - p < 0.001$
ϑ_{Max}	$\uparrow - p < 0.001$	$\downarrow - p < 0.001$
ϑ_{Min}	$\uparrow - p < 0.001$	$\uparrow - p < 0.001$
ϑ_{Mean}	$\uparrow - p < 0.001$	$\uparrow - p < 0.001$
ϑ_{Range}	$\uparrow - p < 0.001$	$\downarrow - p < 0.001$
ψ_{Mean}	$\downarrow - p < 0.001$	$\downarrow - p < 0.001$
Mid-Upstroke α	$\downarrow - p < 0.001$	$\downarrow - p < 0.001$
Mid-Downstroke α	$\downarrow - p = 0.027$	$\downarrow - p < 0.001$
Relative Timing of Supination (t/T)	$\rightarrow - p < 0.001$	$\leftarrow - p < 0.001$
Relative Timing of Downstroke End (t/T)	$\rightarrow - p < 0.001$	$\rightarrow - p < 0.001$
Wingbeat Frequency (Hz)	$\uparrow - p < 0.001$	$\downarrow - p < 0.001$

The kinematic changes brought about by the gear change mechanism will cause a variety of changes to both the magnitude and direction of the force output of the wing (Ellington, 1984a,b; Lehmann and Dickinson, 1998; Sane and Dickinson, 2001; Bomphrey et al., 2005; Dickinson, 2005; Bergou et al., 2010; Jankauski et al., 2017; Chen et al., 2017). The changes observed in the stroke plane between the different gears (figure 3.7), for example, imply that the mean resultant force of the wing will

be directed more vertically during gear 3, but would be tilted more ventrally during gears 1 and 2. This would suggest that gear 3 wing movements may be more suited to vertical ascension, while gears 1 and 2 would be better applied to forward flight. At the same time, the greater stroke amplitude observed in gear 3 wingbeats would suggest that the wing is accelerating a greater volume of air during these wingbeats, which suggests higher aerodynamic force output, just as would be required to lift the body. However, the stroke plane of gear 3 wingbeats is still tilted somewhat nose-down (figure 3.7), and so will produce an anteriorly directed resultant force that can be used for forward flight as well.

Even the subtle changes in the timings of wing rotation that we observe across gears (figure 3.12) will cause significant changes in the force output of the wingbeat. Minute changes in the amount of time spent in different phases of the wingbeat will cause changes in airflow over the wing, and affect wake-capture as well (Dickinson et al., 1999). Similar alterations to wake-capture can also be brought about by changes in the wing pitch angle relative to the direction in which the wing is moving. This is exemplified by the significant differences in the relative timings of supination compared to the timing of the downstroke and upstroke across the different gears.

Differences in the relative timing of wing supination is also a feature shared by changes in alula state in Walker et al.'s (Walker et al., 2012) study. Given this also seems to be a feature of the gear change, this lends weight to the idea that the alula can be used as an indicator of the mechanism. Indeed, many kinematic changes are associated with both gear changes and alula state changes (table 3.7). However, there is no definitive relationship between the gear engaged and the alula state occupied (table 3.2). There is, however, a somewhat dependent relationship (tables 3.3 & 3.4) in that some alula states are significantly more likely to occur at the same time as particular gear engagements, though this is unlikely to be a causal relationship, as any gear-alula combination can be produced. Alula state changes are thought to be indicative of *Ax3* muscle activity (Walker et al., 2012), suggesting that the muscles attached to *Ax3* (Dickinson and Tu, 1997) may play a role in

determining the gear engaged during each wingbeat. The alula can occupy either state regardless of the gear engaged, and so other muscles must also be responsible for determining gear changes themselves.

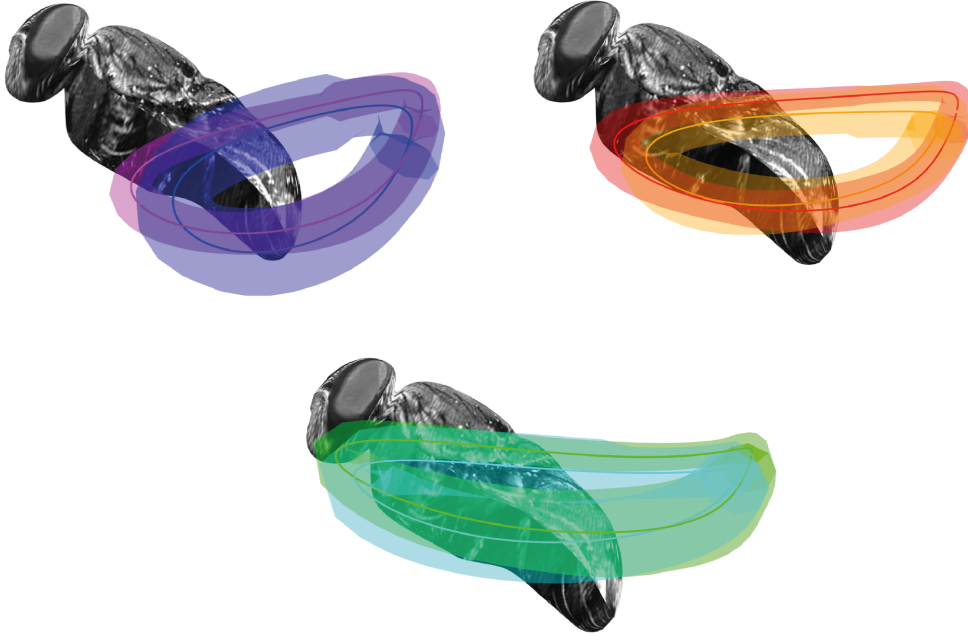


Figure 3.17: Three-dimensional wingtip paths taken during different gear-alula state combinations. The thin bold line represents the mean wingtip path, while the surrounding patch represents one standard deviation from this mean. Top left: gear 1, blue - alula flipped ($n = 929$ wingbeats), magenta - alula flat ($n = 874$). Top right: gear 2, orange - alula flipped ($n = 1,113$), red - alula flat ($n = 224$). Bottom: gear 3, cyan - alula flipped ($n = 2,904$), green - alula flat ($n = 780$); total $n = 6,821$ wingbeats from 15 individuals.

If there is no muscular mechanism linking the state of the alula with the gear engaged during any given wingbeat, there may be another reason that we see individual gears displaying certain alula states more often. The various gear-alula combinations show significantly different kinematics (figure 3.17, table 3.6), and it may be the case that certain sets of kinematics produced by these gear-alula state combinations are used more commonly during flight, leading to our finding that certain alula states are more frequently seen during certain gears.

Interestingly, where Walker et al. (Walker et al., 2012) found earlier wing supination with alula flipping in *E. tenax*, the results presented here in *C. vicina* show significantly earlier supination when the alula is flat (figure 3.16). However, other

kinematic differences that we found to be associated with alula flipping were shared with that of Walker et al., such as a reduced stroke amplitude during flipped-alula wingbeats. The fact that not all of these results were shared suggests that there may be some differences in the mechanism of alula flipping across species, and may be similar differences in the gear change mechanism too. Our results here are consistent with those reported during a study of the gear change mechanism in *Calliphora* by Nalbach (Nalbach, 1989), but more studies of the mechanism in different species, such as *E. tenax* are warranted.

3.5 Conclusions

Wingbeats of *C. vicina* can be classed as different gears depending on whether and how the *RS* and *PWP* interact with each other. Each of these gears exhibit variations in wing kinematics compared to each other, and this will thereby result in different aerodynamic force outputs resulting from these wingbeats. A detailed study of the precise changes in force output as a result of these changes in gear is not possible here, but it is obvious that bilateral asymmetries in gear engagement will also produce asymmetries in force output. The differences in wing kinematics are likely brought about due to the fact that the *RS-PWP* interaction absorbs kinetic energy from the wing. Similarly, the two alula states are also associated with differences in wing kinematics, and flies may engage in a variety of gear-alula state combinations to produce sets of wing kinematics required for whole-body manoeuvres.

Despite similar kinematic differences between both gear changes and changes in alula state there is no definitive relationship between the two. There is, however, a significant relationship, but only to the extent that certain gears are significantly more likely to be associated with certain alula states. This implies that there is no single mechanism controlling both the gear change mechanism and the state of the alula. There may instead be two discrete muscular mechanisms that operate as independent, almost binary switches in which the active/inactive state of certain muscles govern the position of the alula and the engaged gear. This study has not

been able to determine which muscles are responsible, though the muscles attached to *Ax3* have been implicated by previous authors and deserve further study.

Of course it remains a possibility that *RS-PWP* interaction is not strictly required for the control of wing kinematics, and that the control muscles are capable of determining wing kinematics without the gear change mechanism. In which case, the interaction may be a mechanism through which control that would otherwise be exerted by muscular work can instead be performed by *RS-PWP* contact, thereby reducing metabolic energy expenditure. The gear change mechanism could therefore provide a way in which coarse control over wing movement can be efficiently exercised, while the control muscles are responsible for exerting fine control within each gear. This may make the flight motor more efficient while also affording the insect greater control over wing kinematics and manoeuvres. Whether this is the case is outside the scope of this study and will be explored in future work.

The results of this study demonstrate that *C. vicina* makes extensive use of interactions between the *RS* and *PWP* to bring about different sets of wing movements. Mechanical interactions such as these could be utilised to control the magnitude of oscillations in systems such as ornithopters, and future studies and the following chapters will elucidate more details about the biomechanics of this mechanism.

Chapter 4

The Gear Change Mechanism in *Eristalis tenax*

The gear change mechanism is thought to provide a method through which dipteran insects can control their wing movements. We have previously utilised high-speed macrovideography to document the radial stop (*RS*) and pleural wing process (*PWP*) interaction that forms the gear change mechanism and quantify its effects on wing kinematics in *Calliphora vicina*, but it has not yet been possible to determine exactly how the mechanism functions. Here, we document the mechanism in *Eristalis tenax*, and utilise a comparative anatomy approach to infer how the mechanism functions. We also examine movements of the alula to identify any common underlying muscular controls between this and the gear change mechanism. We find that *E. tenax* possesses only two gears, each with a distinct set of wing kinematics. Unlike in *C. vicina*, we see no way in which the restriction of wing motion by *RS-PWP* interaction could cause the wing kinematic changes observed in *E. tenax*. Instead, we assert that it functions to influence wing rotation and stroke reversal at the end of the downstroke. We believe that these differences are due to anatomical variations of the *PWP* between these two species. There is also no definitive relationship between the movement of the alula and the gear change mechanism, implying that there is no common muscular control. This study has further revealed how the gear

change mechanism may act to control wing kinematics in dipteran insects, though future work is required to determine the muscular control of this unique mechanism.

4.1 Introduction

The previous chapter demonstrated that the interaction between the *RS* and *PWP* correlates with wing kinematic changes in *Calliphora vicina*, but it is unclear how this gear change mechanism functions to produce these changes. This contact between these two components is thought to function as a brake, inhibiting wing motion and determining wing kinematics as a result. However, evidence for this function is not conclusive, and the possibility remains that this interaction performs other hypothesised roles, such as being an aid for wing pitch control.

One common methodology used in studying the functionality of anatomical features is comparative anatomy, in which differences in physical characters of various species are used to infer functional explanations for differences in other qualities, such as behaviour or mobility. Fortunately, dipteran insects show a great diversity of morphologies and ecologies across species, with the order being split into two sub-orders, the Nematocera, including the crane flies (Tipulidae) and mosquitoes (Culicidae), and the Brachycera, such as the blowflies (Calliphoridae) and hoverflies (Syrphidae). Across and within these families, Diptera exhibit a wide variety of different morphologies, ecologies, and behaviours. The *RS* and *PWP* are also often present, and their forms differ significantly across species Miyan and Ewing (1985a). We can therefore employ comparative anatomy to help provide functional explanations for the interaction of the two components.

Drone flies, *Eristalis tenax*, exhibit remarkable control over their flight, and as such must be able to control their wing kinematics with extreme finesse. The wing kinematics of this species has been studied previously (Liu and Sun, 2008; Walker et al., 2009, 2010, 2012), but the gear change mechanism has never been examined in any detail. Given the differences between *E. tenax* and previously studied species such as *C. vicina* (Pfau, 1973, 1987; Ennos, 1987; Wisser, 1987, 1988; Nalbach, 1989)

and *Parasarcophaga dux* (Deora et al., 2015) both in terms of anatomy and flight capability (figure 4.2), examining the gear change mechanism of *E. tenax* in detail is likely to provide new insight into the function of *RS-PWP* interaction.

One recent study of free flight of *E. tenax* also revealed wing kinematic changes reminiscent of the gear change mechanism occurring with movement of the alula (Walker et al., 2012), just as we found in *C. vicina* in Chapter 3. Though we failed to find a definitive relationship between the state of the alula on a given wingbeat and the gear engaged in this species, we did find a propensity for differently geared wingbeats to exhibit certain alula states. If a similar relationship can be identified in *E. tenax* it may reveal more information about the underlying muscular control of the gear change mechanism.

In this study, we have filmed the gear change mechanism of *E. tenax* and documented movements of the alula during the wingbeat in real time. Using photogrammetry, we have also tracked the wing kinematics during these different gear engagements and alula states. We then compare the findings of this study to that of Chapter 3, and use this to learn more about the role of the gear change mechanism in the dipteran flight motor, the biomechanics through which it functions, and the potential relationship between the mechanism and the movement of the alula identified by previous authors. We see a number of differences in the mechanism between these two species, which appear to relate to anatomical dissimilarities resulting in differential abilities to absorb energy and inhibit wing movement. Despite this, the gear change mechanism significantly affects wing kinematics in both species, but does so in different manners. However, we fail to find any evidence for a mechanical relationship between the alula and the gear change mechanism. This study has continued to further our comprehension of the gear change mechanism and its function, but understanding the underlying muscular control requires further work. As we continue to understand more about this mechanism, we get closer to being able to replicate it or draw upon it for inspiration in novel engineering applications, such as the design of micro- or nano-air vehicles.

4.2 Materials and Methods

4.2.1 Insect Preparation

We caught wild *E. tenax* individuals from the Oxford area and brought them to the lab. Within the lab, we kept the insects in an insect rearing tent (Watkins and Doncaster Bugdorm-43030), providing them with a sugar water mixture, dry bee pollen (Supernutrients Bee Pollen, Holland & Barrett, Nuneaton, Warwickshire, UK), and wetted bee pollen for nutrition and hydration. The insects were kept out of the view of external windows to avoid them attempting to escape their enclosures and damaging their wings in the process. The lab itself was under ambient light conditions, and at a temperature between 20-22°C.

Before tethering, we cold anaesthetised the insects at between 3-5°C for between 3 and 5 minutes depending on the size of the insect, until it was completely immobilised, and placed it in a small groove cut into a sponge. We then placed a small amount of a heated mixture of beeswax and colophonium onto the scutum of the insect, and lightly pressed a plastic rod against this to tether the insect to the rod itself.

4.2.2 High-Speed Camera Set Up

We positioned four high-speed cameras (two Photron FASTCAM Mini AX100 type 800K-M-16G cameras, and two Photron FASTCAM SA3 model 120K-M2 cameras, Photron Ltd.) to film both the wing and the wing hinge in the same way as in Chapter 3, including the same flight stimulus of a fan. In a variation to the previous work, we used a custom-built LED array to illuminate the wing being filmed by the two SA3 cameras positioned above the insect. We fitted this array with a 3mm layer of semitransparent acrylic to diffuse the light equally across the array, and cut a small groove in the acrylic into which we inserted the plastic tether to which the insect was attached. The insect and its wings were therefore backlit by the LED array below it.

4.2.3 Three Dimensional Wing Kinematics Measurement

The calibration procedure and wing kinematic measurements used in this study are the same as those utilised in Chapter 3, with the exception that it was not possible to resolve the shape of the wing, and wing pitch angle has therefore not been measured.

4.2.4 Wingbeat Classifications

After filming the base of the wing during flight, we classified each wingbeat as gear 1 or 3 depending on whether the *RS* and *PWP* interacted during the wingbeat. In keeping with the nomenclature of the gear change mechanism in *Calliphora* (Chapter 2, Nalbach (1989)), we defined gear 3 as a wingbeat in which the *RS* and *PWP* did not come into contact at all, and gear 1 as a wingbeat in which the *RS* contacted the groove on the head of the *PWP*. As there is only one groove in the head of the *PWP* in *E. tenax*, there is only type of *RS-PWP* interaction, and so we have omitted gear 2 from this nomenclature. Finally, we defined gear 0 as a wingbeat in which the *RS* locked behind the *PWP*, such that the *RS* never moved dorsally of the *PWP* as it does in other gears, as was observed in *C. vicina* in Chapter 3. However, this gear was never observed in *E. tenax*.

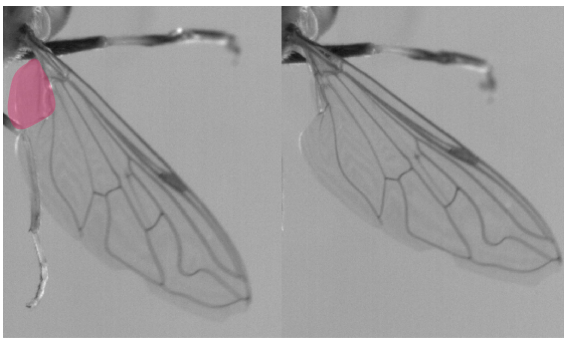


Figure 4.1: Images of the *E. tenax* wing during tethered flight showing the two states of the alula. Left: An alula in its flat state (highlighted in red). Right: An alula in its flipped state.

In regards to the state of the alula, we examined the wing using the three videos of the whole wing to identify whether or not the alula was flat or flipped. This was generally clearest at the point of supination at the end of the downstroke. If the alula was flat to the wing, it was clearly visible, whereas it was not visible if it was flipped (figure

4.1). In the event that the state was not clear, either due to the alula appearing to be in an intermediate state, or due to obfuscation by the body or legs, this wingbeat was not included in any analyses relating to alula state.

4.2.5 SEM Image Collection

We took individuals of both *C. vicina* and *E. tenax* and froze them before drying the specimens. We then performed the same SEM image collection procedure as outlined in Chapter 2.

4.2.6 Statistical Analysis

We performed all our statistical analyses and data manipulation using MATLAB (The Mathworks Inc., Natick, MA, USA). To determine statistically significant differences amongst the kinematics of wingbeats in different gears and alula states, we used two-sample t-tests with an α -value of 0.05.

After classing each wingbeat according to its gear engagement and alula state, we used χ^2 tests to observe any significant relationship between alula-flipping behaviour and *RS-PWP* interaction. To determine where any significant relationships stemmed from within the contingency table of gear engagement and alula states, we calculated adjusted residuals (r_{adj}) between the expected and observed values using the following equation:

$$r_{adj} = \frac{O - E}{\sqrt{E \times (1 - \frac{Rt}{n}) \times (1 - \frac{Ct}{n})}} \quad (4.1)$$

Where O is the observed value, E is the expected value, Rt is the row total for that alula state, Ct is the column total for that gear state, and n is the total number of observations.

As described in Chapter 3, we applied a false discovery rate algorithm to each individual set of comparisons (the across-gear state and across-alula state kinematic comparisons are separate for this purpose) to compensate for the large number of tests being performed and reduce the risk of type I errors.

4.3 Results

4.3.1 *RS* and *PWP* Anatomy

The SEM images shown in figure 4.2 show the striking differences in the anatomy of the wing hinge components between *C. vicina* and *E. tenax*. The *PWP* of *E. tenax* is much smaller than that of *C. vicina*, being approximately 92µm and 68µm in height and width in the former, and 149µm in height and 134.6µm in width in the latter (measured from figure 4.2). The *PWP* also possesses two grooves rather than just one. This difference is particularly notable given that *E. tenax* is a larger species, with a wingspan of 10-12mm compared to a wingspan of between 8-9mm in *C. vicina*. The *PWP* of *E. tenax* is therefore both absolutely smaller, and smaller relative to the body size compared to *C. vicina*.

The *RS*, on the other hand, is very similar between the two species. Both have a cup on the anterior side, into which the Pterale C (*PtC*) fits during the latter half of the downstroke (see supplementary videos 3.1 to 3.5, and 4.1 to 4.2), and both are roughly the same pointed, prong-like shape which fits into the grooves on the head of the *PWP*. Despite the differences between these two components, the similarities between them are sufficient to suggest that they perform similar roles in both species compared here.

4.3.2 Types of *RS-PWP* Interaction

The gear change mechanism has never been documented before in *E. tenax*. We obtained recordings of 5,563 wingbeats from 11 individuals, which reveal that this species only utilises two gears during flight, compared to the three observed in *C. vicina* (supplementary videos 4.1 and 4.2, demonstrating gear 1 and gear 3 wingbeats, respectively; Chapter 3, (Pfau, 1973; Nalbach, 1989)). This is down to the anatomy of the *PWP* which possesses only one groove, compared to the two grooves in the head of the *C. vicina* *PWP*, meaning that there is only one configuration in which the *RS* can engage with a groove of the *PWP* in *E. tenax* (figure 4.2), as has

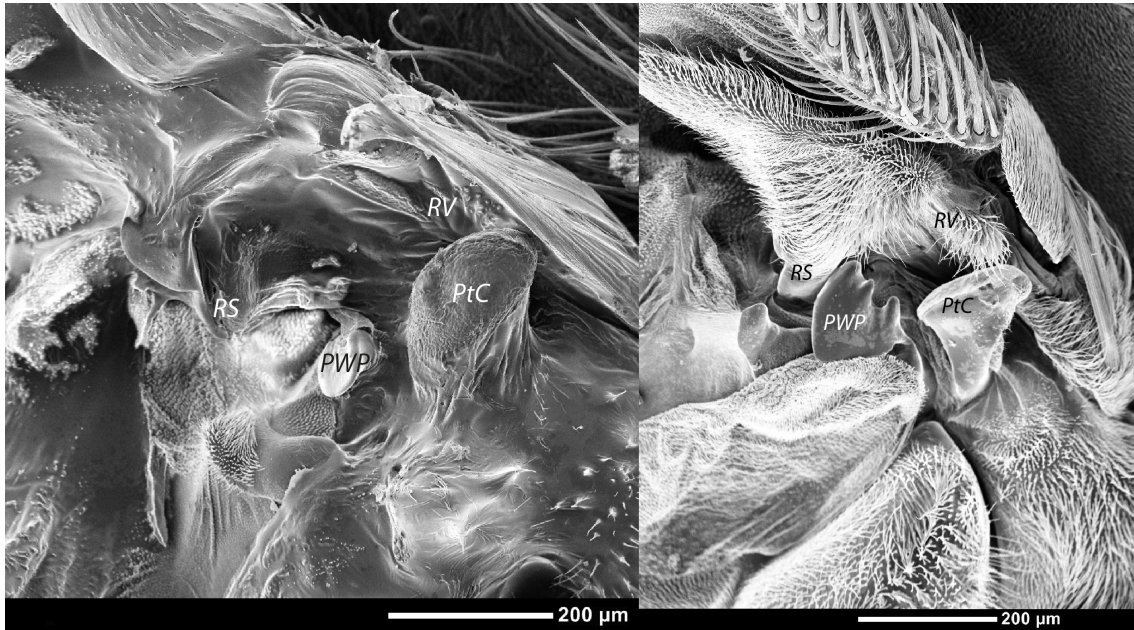


Figure 4.2: Scanning electron microscope images of *E. tenax* (left) and *C. vicina* (right) right wing hinges, including the components involved in the gear change mechanism. In the right panel, the *RS* has locked behind the *PWP* in a gear 0 configuration. Note the difference in the numbers of grooves in the *PWP* of both species: one in *E. tenax* (left) and two in *C. vicina* (right). Abbreviations: *PtC* - Pterale C; *PWP* - Pleural Wing Process; *RS* - Radial Stop; *RV* - Radial Vein.

been suggested previously by (Miyan and Ewing, 1985a). Of these wingbeats, 1,452 were classed as being in gear 1, 4,107 were classed as gear 3, and 4 wingbeats were not classifiable due to the gear state being unclear. Gear 0 wingbeats, which are typified by severely impeded wing motion and during which the *RS* locks behind the *PWP*, were never observed in this study.

It was far more difficult to discern the engagement of the *RS* and *PWP* compared to *C. vicina*. This stems in part from differences in the size of the *PWP* relative to the *RS* between these two species (the *PWP* and its grooves are far smaller relative to the *RS* in *E. tenax* compared to *C. vicina*), but it is also due to differences in the motion of these components around the time of engagement. Furthermore, unlike in *C. vicina*, we did not observe any sort of jarring, flicking motion of the *RS* upon disengagement from the *PWP* components in during *E. tenax* flights (supplementary video 4.2).

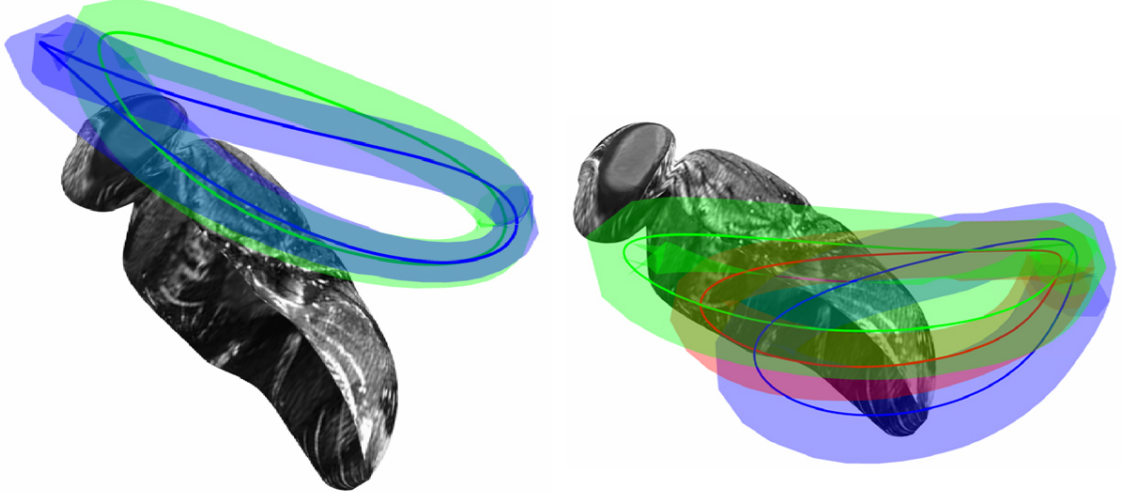


Figure 4.3: Three-dimensional paths of the wingtips across different gears in *E. tenax* (left) and *C. vicina* (right). Note that the stroke plane of *E. tenax* is much higher than in *C. vicina*. *E. tenax* displays only two gears (green - gear 3, blue - gear 1), while *C. vicina* shows three (green - gear 3, red - gear 2, blue - gear 1). In *E. tenax*, gear 3 is determined by no *RS-PWP* interaction, while in gear 1 the *RS* engages with the groove of the *RS*. In *C. vicina*, gear 3 wingbeats show no interaction, while gears 2 and 1 show *RS* engagement with the anterior and posterior grooves of the *PWP*, respectively. Note that differences in body angle are representative of the body positions experienced by the insects during tethered flight. $n = 5,559$ for *E. tenax*, and $n = 6,821$ for *C. vicina*.

4.3.3 Wing Kinematic Differences Between Gears

Between the two gears observed here, we have found significant differences between the kinematics of the wings across the wingbeat. Figure 4.3 shows the three-dimensional paths taken by the wingtips during these differently geared wingbeats, which illustrates that gear 3 wingbeats (in which there is no *RS-PWP* contact) showed a somewhat more restricted path compared to wingbeats in gear 1 (where the *RS* engaged with the *PWP*). Both the posterior position of the wingtip at the end of the upstroke and the anterior wingtip position at the end of the downstroke were different between the two wingbeats, with gear 1 wingbeats reaching greater anterior position than gear 3 wingbeats at the end of the downstroke, while gear 3 wingbeats reached a greater posterior position at the end of the upstroke than gear 1 wingbeats (figures 4.3 & 4.4, table 4.1).

These differences in wingtip positions are best illustrated by the significant differences found between the maximum stroke angle of wingbeats in gears 1 and 3 ($t_{5557} = 39.56$, $p < 0.001$), as well as the variation in minimum stroke angle between

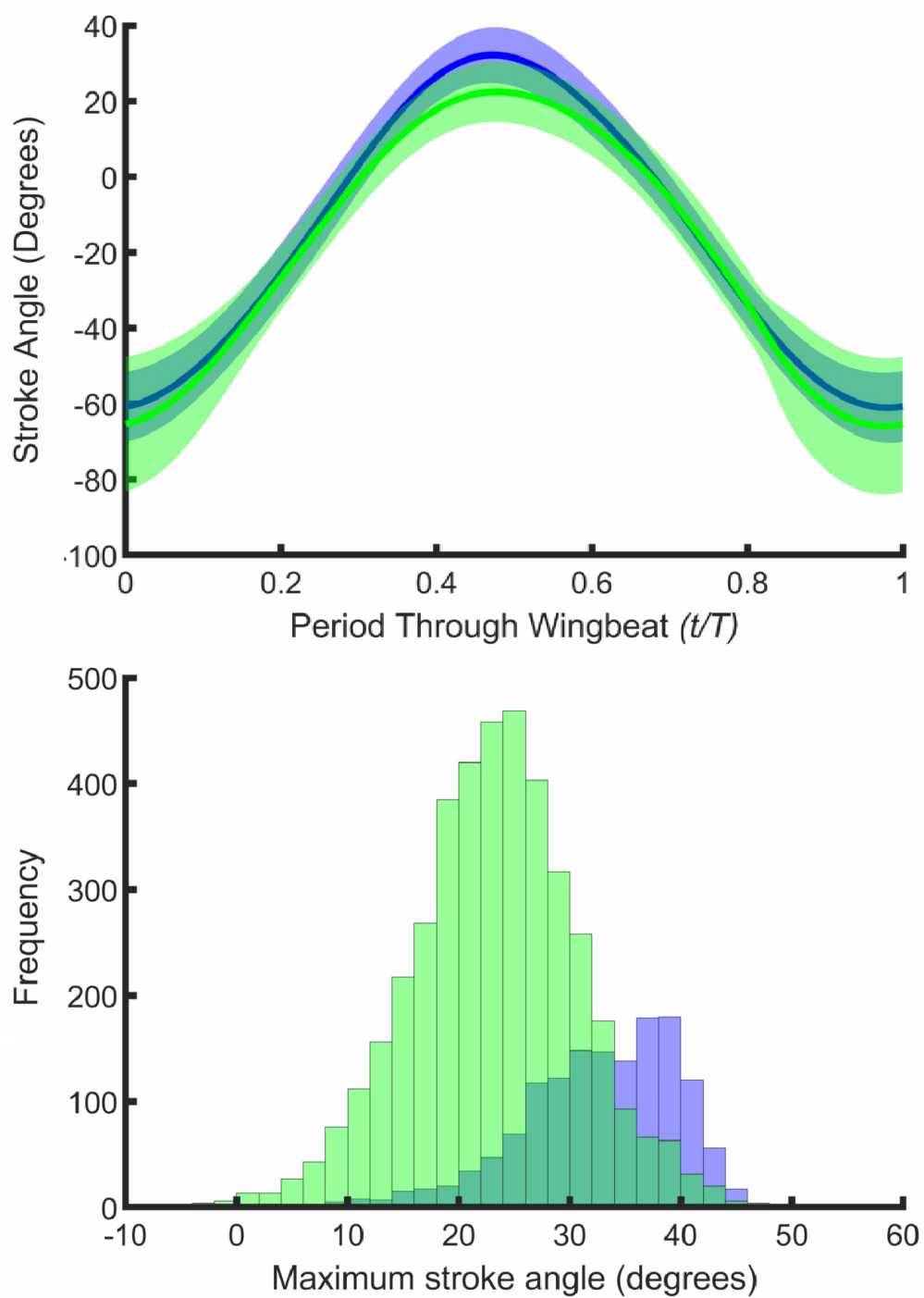


Figure 4.4: Top: Stroke angle plotted over the course of both gear 1 (blue, $n = 1452$) and gear 3 (green, $n = 4107$) wingbeats. The thin, bold line shows the mean, while the surrounding translucent patch shows one standard deviation from this mean. Bottom: a histogram of maximum stroke angles during both gear 1 (blue, $n = 1452$) and gear 3 (green, $n = 4107$) wingbeats. t = time and T is the total duration of the wingbeat, where t is equal to zero at the start of the downstroke.

the two (figure 4.4; $t_{5557} = 11.42$, $p < 0.001$). Stroke amplitude (the range in stroke angle between the ends of the downstroke and upstroke) therefore also significantly varied between gear 1 and gear 3 wingbeats ($t_{5557} = 39.56$, $p < 0.001$). These results demonstrate that gear 3 wingbeats show a smaller range of movement in terms of stroke angle than gear 1 wingbeats (figure 4.4). This is unusual, as if *RS-PWP* contact acts as a braking mechanism to inhibit anterior wing motion (as has been hypothesised and appears to be the case in *C. vicina*, see Chapter 3), we might expect that gear 1 wingbeats would be the more restricted modes of movement.

Significant differences are present again as we examine stroke deviation angle across the two gears (figure 4.5 & table 4.1). Gear 1 wingbeats show a lower mean deviation angle compared to gear 3 wingbeats ($t_{5557} = 10.14$, $p < 0.001$), resulting in part from gear 3 wingbeats exhibiting the largest maximum deviation angle of the two gears ($t_{5557} = 23.64$, $p < 0.001$). However, gear 3 wingbeats also display a greater minimum deviation angle than gear 1 wingbeats ($t_{5557} = 11.17$, $p < 0.001$). Wingbeats in gear 3 thereby also show the largest range of deviation angles ($t_{5557} = 23.37$, $p < 0.001$). The stroke plane around which this deviation occurs is also significantly different between the two gears, with gear 1 wingbeats showing a more declined stroke plane angle than those of gear 3 ($t_{5557} = 3.416$, $p < 0.001$).

There is further disparity between the gears as we examine the differences in the timing of the end of the downstroke, with downstroke finishing significantly earlier during gear 1 wingbeats compared to gear 3 wingbeats ($t_{5557} = 15.91$, $p < 0.001$). Despite this difference, there is a large amount of overlap in the time of the downstroke end across both gears (figure 4.6). It is interesting to note that gear 1 wingbeats show a smaller interquartile range ($t/T = 0.0249$, where t is time and equal to zero at the start of the downstroke, while T is the total duration of the wingbeat), than gear 3 wingbeats ($t/T = 0.0383$), which suggests that *RS-PWP* engagement might be used to restrict wing kinematics to a certain range.

Finally, we see no significant differences between the wingbeat frequencies between the different gears, where both gear 1 and gear 3 wingbeats exhibited very

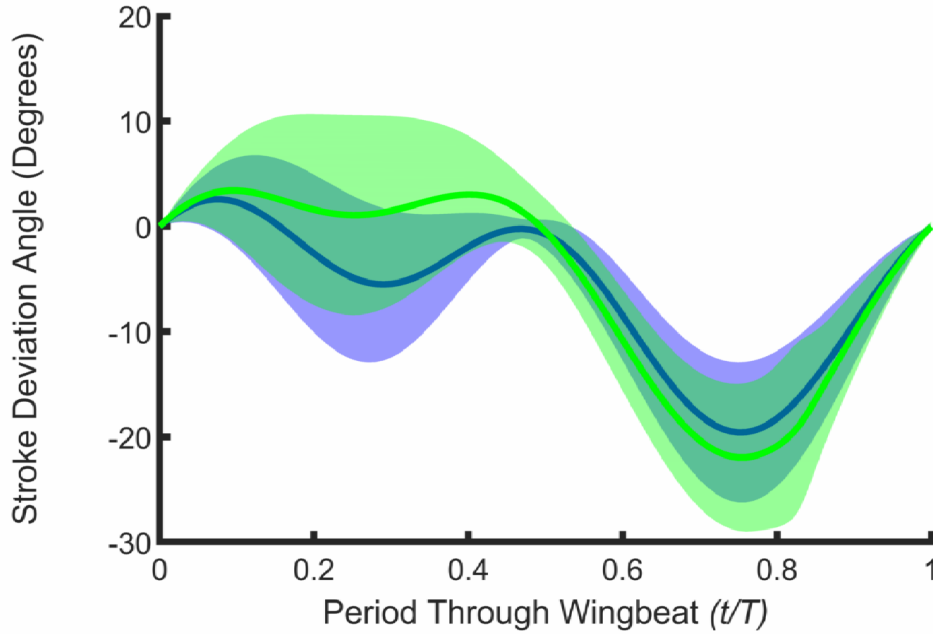


Figure 4.5: Stroke deviation angle plotted over the course of both gear 1 (blue, $n = 1452$) and gear 3 (green, $n = 4107$) wingbeats. The thin, bold line shows the mean, while the surrounding translucent patch shows one standard deviation from this mean.

similar wingbeat frequencies across recordings ($t_{5557} = 0.145$, $p = 0.885$).

4.3.4 The Alula as an Indicator of the Gear Change Mechanism

Our observations from the video recordings reveal that the alula can occupy either a flat or a flipped state regardless of the state of the gear change mechanism (table 4.2). We only observed one wingbeat in which we could not confidently discern the state of the alula (of 5,563 wingbeats). However, a χ^2 test reveals a significant relationship between the state of the alula and the gear change mechanism ($\chi = 240.6$, $p < 0.001$).

The adjusted residuals found in table 4.3 indicate that, gear 1 wingbeats also showed proportionally more occasions in which the alula was flat, while gear 3 wingbeats show proportionally more wingbeats in which the alula flipped. We can conclude from this that, while the alula cannot be used as a precise indicator for the state of the gear change mechanism, we can use it to make predictions. For

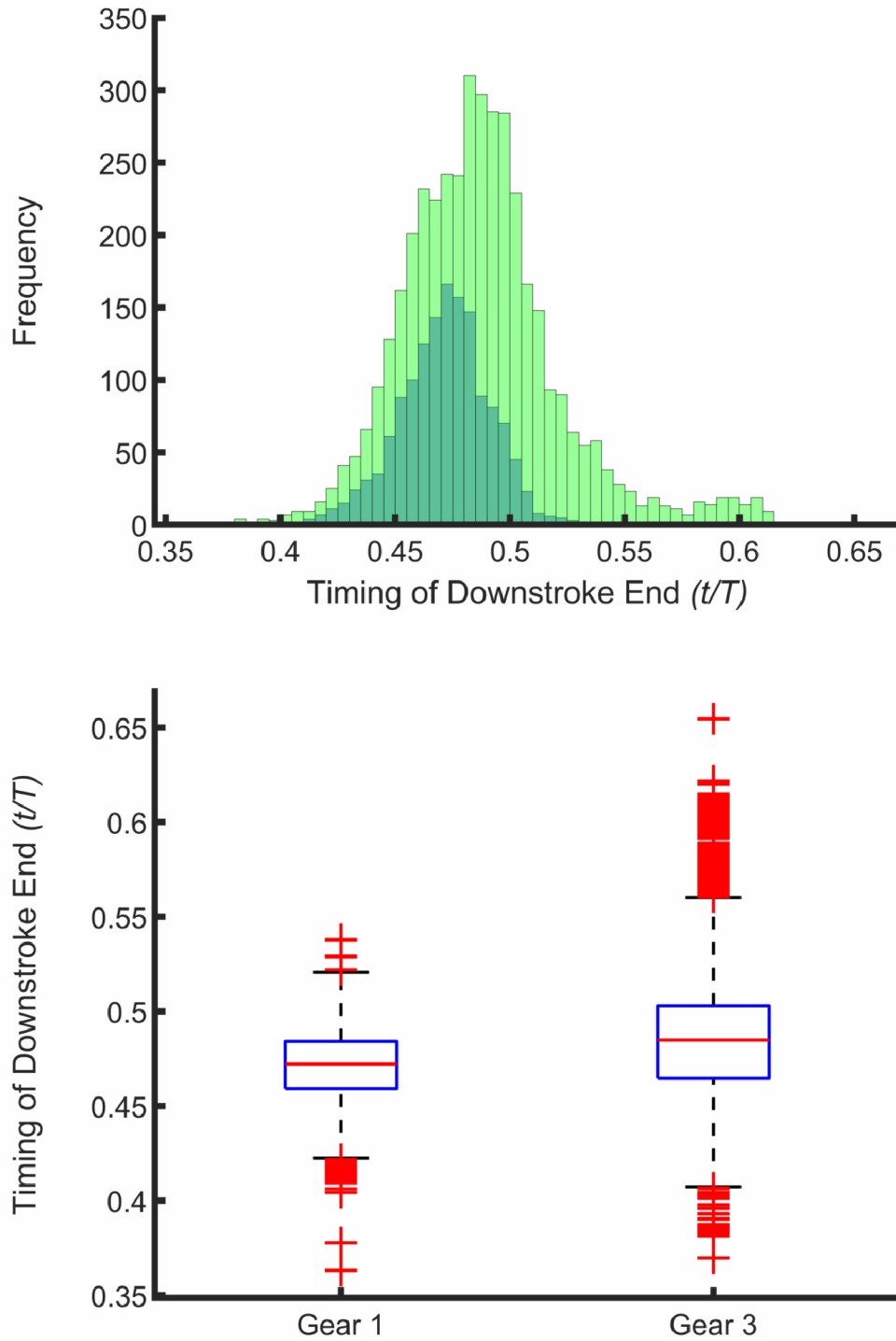


Figure 4.6: Top: A histogram displaying the variation in the timing of the end of the downstroke across both gear 1 (blue bars, $n = 1452$) and gear 3 (green bars, $n = 4107$). Bottom: Box and whisker plots of the timing of the end of the downstroke across both gears, showing the range (black bars), interquartile range (blue box), median (red bar) and outliers (red crosses).

Table 4.1: Kinematics of the wings during different gears of *E. tenax*, presented as the mean $\pm$ one standard deviation and the resultant t - and p -values resulting from a two sample t -test. Bold values indicate significant results after the application of a false discovery rate algorithm. φ = stroke angle (φ_{Range} therefore represents stroke amplitude), ϑ = deviation angle, α = wing pitch angle, ψ = stroke plane angle.

Kinematic	Gear 1	Gear 3	Significance
φ_{Max}	$32.61 \pm 7^\circ$	$23.27 \pm 8^\circ$	$t_{5557} = 39.56$, $p < 0.001$
φ_{Min}	$-62.42 \pm 10^\circ$	$-68.64 \pm 20^\circ$	$t_{5557} = 11.42$, $p < 0.001$
φ_{Range}	$93.39 \pm 11^\circ$	$88.45 \pm 19^\circ$	$t_{5557} = 9.312$, $p < 0.001$
ϑ_{Max}	$3.688 \pm 2^\circ$	$6.94 \pm 5^\circ$	$t_{5557} = 23.64$, $p < 0.001$
ϑ_{Min}	$-21.46 \pm 6^\circ$	$-24 \pm 8^\circ$	$t_{5557} = 11.17$, $p < 0.001$
ϑ_{Mean}	$-6.174 \pm 2^\circ$	$-5.267 \pm 3^\circ$	$t_{5557} = 10.14$, $p < 0.001$
ϑ_{Range}	$25.41 \pm 6^\circ$	$30.94 \pm 9^\circ$	$t_{5557} = 23.37$, $p < 0.001$
ψ_{Mean}	$-17.2 \pm 6^\circ$	$-16.34 \pm 9^\circ$	$t_{5557} = 3.416$, $p = 0.006$
Relative Timing of Downstroke End (t/T)	0.471 ± 0.02	0.487 ± 0.04	$t_{5557} = 15.91$, $p < 0.001$
Wingbeat Frequency (Hz)	144.8 ± 11	144.8 ± 10	$t_{5557} = 0.145$, $p = 0.885$

Table 4.2: Frequency counts of the number of wingbeats in different gear-alula state combinations.

	Gear State		
Alula State	<i>Gear 1</i>	<i>Gear 3</i>	Total
<i>Flat</i>	600	845	1445
<i>Flipped</i>	851	3262	4113
Total	1451	4107	5558

Table 4.3: Adjusted residuals derived from the results in table 4.2. Statistically significant values are in bold.

	Gear State	
Alula State	<i>Gear 1</i>	<i>Gear 3</i>
<i>Flat</i>	15.51	-15.51
<i>Flipped</i>	-15.51	15.51

example, an alula-flipped wingbeat is far more likely to be engaged in gear 3 than gear 1.

4.3.5 Wing Kinematic Differences between Alula States

The relationships we observed between the state of the alula and the gear engaged on any particular wingbeat also extends to the differences we observe in wing kinematics between alula states (figure 4.7 & table 4.4). Like the dichotomy we saw between the two different gears (figure 4.3), the positions of the wingtip at the end of the downstroke differed depending on the state of the alula. However, unlike the comparison between gears, the position of the wingtip at the end of the upstroke are much more similar across alula states.

These differences are best illustrated by the examining the stroke angle patterns of the wing during these different alula state wingbeats (figure 4.8). Alula-flat wingbeats exhibited a significantly larger maximum stroke angle than alula-flipped wingbeats ($t_{5560} = 32.5$, $p < 0.001$), but there was no change in minimum stroke angles of the two alula state wingbeats ($t_{5560} = 2.13$, $p = 0.032$; this was deemed non-significant after the application of a false discovery rate algorithm upon the data presented here). Accordingly, the stroke amplitude of alula-flat wingbeats was the greatest of the two alula states wingbeats ($t_{5560} = 13.85$, $p < 0.001$).

Stroke deviation angle also showed significant discrepancies between alula states (figure 4.9 & table 4.4). The mean deviation angle of alula-flat wingbeats is lower

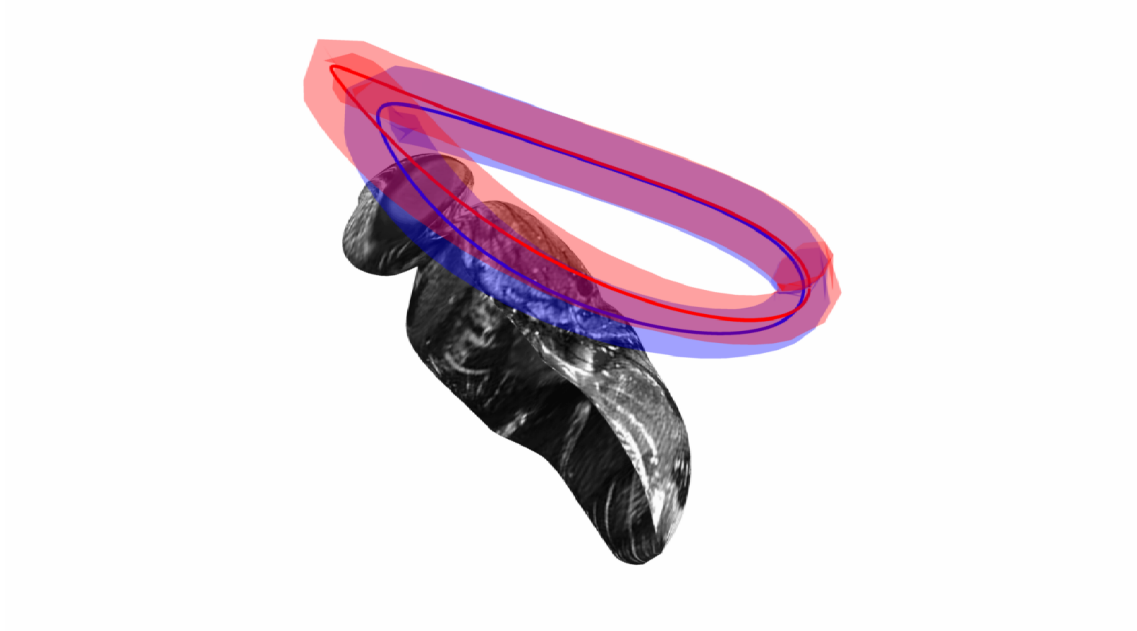


Figure 4.7: Three-dimensional paths of the wingtips across different alula states in *E. tenax*. Red - alula flat ($n = 1445$), blue - alula flipped ($n = 4117$). $n = 5,562$.

than those where the of alula was flipped ($t_{5560} = 17.51$, $p < 0.001$), but we found no significant difference between the minimum deviation angle of alula-flat and alula flipped wingbeats ($t_{5560} = 0.453$, $p = 0.651$). However, we also found that alula-flipped wingbeats displayed a significantly greater maximum deviation angle than alula-flat wingbeats ($t_{5560} = 26.22$, $p < 0.001$). In turn, this results in the greater range of stroke deviation angles seen during alula-flipped versus alula-flat wingbeats ($t_{5560} = 8.393$, $p < 0.001$).

We continue to find variation as we examine the stroke plane, for which alula-flat wingbeats exhibited a more declined stroke plane compared to alula-flipped wingbeats ($t_{5560} = 13.39$, $p < 0.001$). The timing of the end of the downstroke differed significantly too, with the downstroke finishing significantly earlier during alula-flat compared with alula-flipped wingbeats ($t_{5560} = 12.65$, $p < 0.001$). Finally, and unlike between different *E. tenax* gears, there were differences in wingbeat frequencies across the different alula states, in which alula-flat wingbeats showed significantly higher wingbeat frequencies than alula-flipped wingbeats ($t_{5560} = 14.33$, $p < 0.001$).

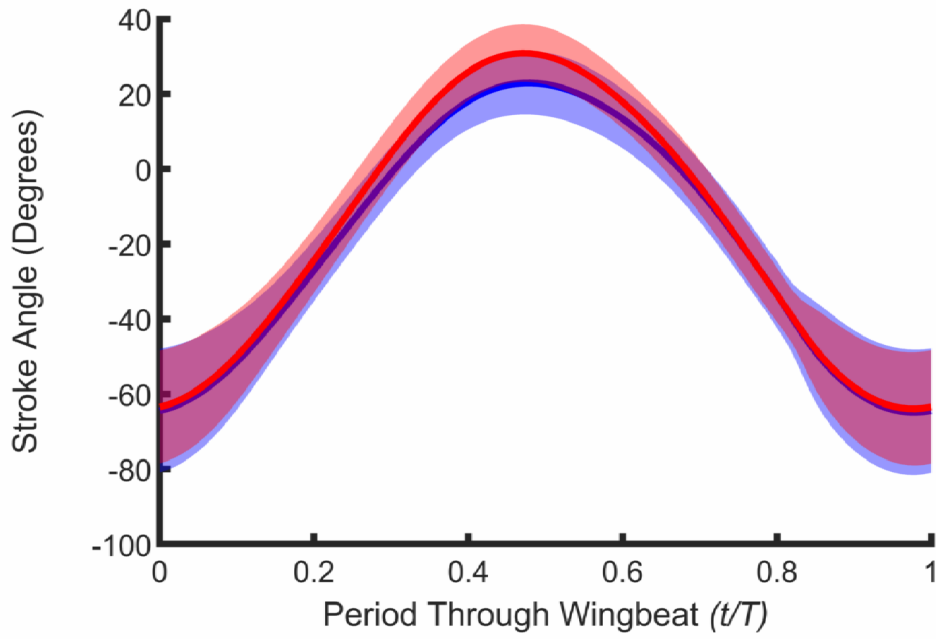


Figure 4.8: Stroke angle plotted over the course of both alula-flat (red, $n = 1445$) and alula-flipped (blue, $n = 4117$) wingbeats. The thin, bold line shows the mean, while the surrounding translucent patch shows one standard deviation from this mean.

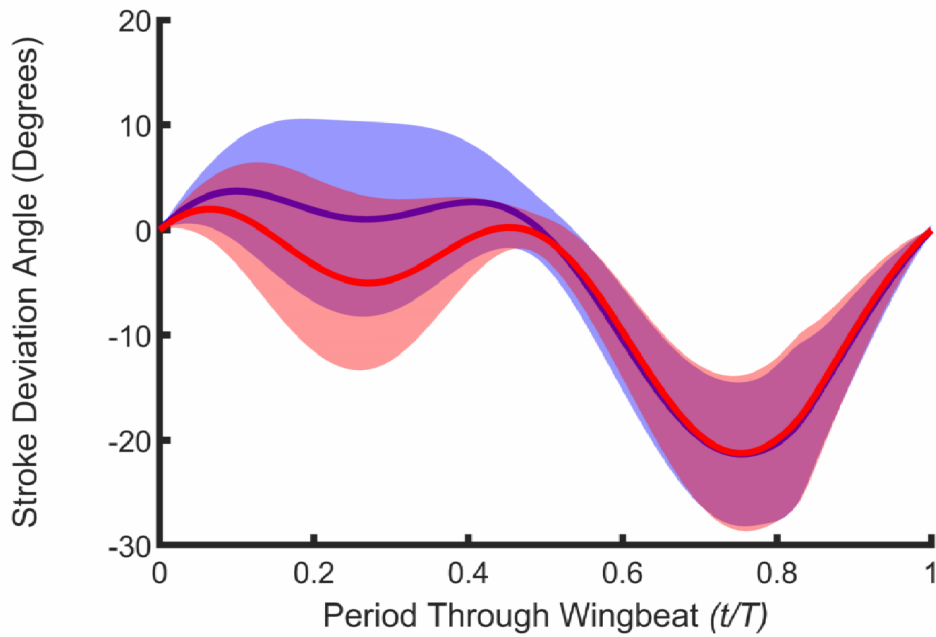


Figure 4.9: Stroke deviation angle plotted over the course of both alula-flat (red, $n = 1445$) and alula-flipped (blue, $n = 4117$) wingbeats. The thin, bold line shows the mean, while the surrounding translucent patch shows one standard deviation from this mean.

Table 4.4: Kinematics of the wings during different alula states of *E. tenax*, presented as the mean $\pm$ one standard deviation and the resultant t - and p -values resulting from an two sample t-test. Bold values indicate significant results after the application of a false discovery rate algorithm. φ = stroke angle (φ_{Range} therefore represents stroke amplitude), ϑ = deviation angle, α = wing pitch angle, ψ = stroke plane angle.

Kinematic	Flat	Flipped	Significance
φ_{Max}	$31.61 \pm 7^\circ$	$23.63 \pm 8^\circ$	$t_{5560} = 32.5$, $p < 0.001$
φ_{Min}	$-66.12 \pm 16^\circ$	$-67.29 \pm 19^\circ$	$t_{5560} = 2.13$, $p = 0.032$
φ_{Range}	$94.98 \pm 15^\circ$	$87.85 \pm 18^\circ$	$t_{5560} = 13.85$, $p < 0.001$
ϑ_{Max}	$3.446 \pm 3^\circ$	$7.019 \pm 5^\circ$	$t_{5560} = 26.22$, $p < 0.001$
ϑ_{Min}	$-23.4 \pm 8^\circ$	$-23.3 \pm 8^\circ$	$t_{5560} = 0.453$, $p = 0.651$
ϑ_{Mean}	$-6.641 \pm 2^\circ$	$-5.1 \pm 3^\circ$	$t_{5560} = 17.51$, $p < 0.001$
ϑ_{Range}	$26.85 \pm 8^\circ$	$30.32 \pm 8^\circ$	$t_{5560} = 8.393$, $p < 0.001$
ψ_{Mean}	$-19.01 \pm 9^\circ$	$-15.7 \pm 8^\circ$	$t_{5560} = 13.39$, $p < 0.001$
Relative Timing of Downstroke End (t/T)	0.474 ± 0.03	0.486 ± 0.03	$t_{5560} = 12.65$, $p < 0.001$
Wingbeat Frequency (Hz)	148 ± 10	143.7 ± 10	$t_{5560} = 14.33$, $p < 0.001$

These many differences between alula states are also retained when taking the state of the gear change mechanism into account. Table 4.5 shows that, while the variations in wing kinematics between alula states are not always as pronounced in certain gear states, the majority of wing kinematics retain significant differences depending on whether the alula is flat or flipped regardless of the gear engaged. Furthermore, the effect of alula flipping appears to be the same regardless of which gear is engaged. Alula-flipping, for example, significantly reduces the stroke amplitude of the wingbeat in both gears 1 and 2 ($t_{1449} = 4.456$, $p < 0.001$; $t_{4105} = 11.37$, $p < 0.001$; for gears 1 and 3, respectively), suggesting that the muscular mechanism controlling the movement of the alula have the same effects on wing motion independent of the mechanism governing the state of *RS-PWP* interaction.

Table 4.5: Comparisons of wing kinematics between different alula states within each gear, presented as the mean $\pm$ one standard deviation, and the resultant t - and p - values resulting from a two-sample t-test. Bold values indicate significant results after the application of a false discovery rate algorithm. φ = stroke angle (φ_{Range} therefore represents stroke amplitude), ϑ = deviation angle, ψ = stroke plane angle.

Kinematic	Gear	Alula State		Significance
		Flat	Flipped	
φ_{Max}	1	35.05 ± 5	30.89 ± 8	$t_{1449} = 11.44$, $p < 0.001$
	3	29.17 ± 7	21.75 ± 7	$t_{4105} = 26.12$, $p < 0.001$
φ_{Min}	1	-61.45 ± 8	-63.09 ± 11	$t_{1449} = 3.224$, $p = 0.001$
	3	-69.43 ± 20	-68.43 ± 20	$t_{4105} = 1.299$, $p = 0.194$
φ_{Range}	1	94.94 ± 10	92.28 ± 11	$t_{1449} = 4.456$, $p < 0.001$
	3	95 ± 17	86.85 ± 19	$t_{4105} = 11.37$, $p < 0.001$
ϑ_{Max}	1	2.807 ± 2	4.312 ± 3	$t_{1449} = 12.36$, $p < 0.001$

	3	3.899 ± 3	7.727 ± 5	$t_{4105} = 20.66,$ $p < 0.001$
ϑ_{Min}	1	20.91 ± 7	21.83 ± 5	$t_{1449} = 2.997,$ $p = 0.003$
	3	25.18 ± 8	23.7 ± 8	$t_{4105} = 4.794,$ $p < 0.001$
ϑ_{Mean}	1	-6.245 ± 2	6.12 ± 2	$t_{1449} = 12.36,$ $p < 0.001$
	3	-6.923 ± 3	-4.838 ± 3	$t_{4105} = 20.66,$ $p < 0.001$
ϑ_{Range}	1	23.72 ± 7	26.14 ± 5	$t_{1449} = 7.561,$ $p < 0.001$
	3	29.08 ± 9	31.43 ± 9	$t_{4105} = 7.025,$ $p < 0.001$
ψ_{Mean}	1	-17.83 ± 8	-16.76 ± 5	$t_{1449} = 3.15,$ $p = 0.002$
	3	-19.85 ± 10	-15.43 ± 8	$t_{4105} = 13.33,$ $p < 0.001$
Relative Timing of Downstroke End (t/T)	1	0.471 ± 0.02	0.472 ± 0.02	$t_{1449} = 0.302,$ $p = 0.763$
	3	0.475 ± 0.04	0.49 ± 0.03	$t_{1449} = 10.94,$ $p < 0.001$
Wingbeat Frequency (Hz)	1	145.2 ± 12	144.5 ± 10	$t_{1449} = 1.201,$ $p = 0.23$
	3	150 ± 9	143.5 ± 10	$t_{4105} = 17.93,$ $p < 0.001$

4.4 Discussion

4.4.1 The Role of the Gear Change Mechanism in *Eristalis tenax*

The role of *RS-PWP* contact in *E. tenax* does not appear to conform with previous hypotheses that the interaction inhibits wing movement and acts as a brake-like mechanism (Wisser, 1988; Nalbach, 1989; Wisser and Nachtigall, 1991; Deora et al., 2015). Past authors have suggested that the role of the gear change mechanism in dipteran insects is to control the stroke angle by impeding wing movement, but this is not apparent in the results presented here. The maximum stroke angles of gear 1 wingbeats (in which the *RS* and *PWP* make contact) are, on average, greater than gear 3 wingbeats (where there is no *RS-PWP* contact; figure 4.4), meaning that there is no suggestion that the interaction mechanically restricts anterior wing movement.

Instead, our findings here corroborate other ideas regarding the role of the mechanism, specifically that the interaction supports wing rotation and stroke reversal (Pfau, 1987; Ennos, 1987; Wisser, 1987). Wingbeats in which there was *RS-PWP* interaction ended the downstroke proportionally earlier in the wingbeat when compared to wingbeats in which the components did not engage with each other. Furthermore, despite there being significant overlap between the downstroke end timings of both gears, the range of values is more restricted in gear 1 compared to gear 3 (figure 4.6), suggesting that, despite not being necessary to bring about the wing movements seen, *RS-PWP* interaction may be utilised to control this movement with more precision. The gear change mechanism may therefore be used to manipulate the aerodynamic effects associated with advances or delays in stroke reversal relative to the pitch angle of the wing. This includes the phenomenon of wake capture, through which the lift force generated by the wing can be enhanced by advancing the wing supination relative to stroke reversal at the end of the downstroke (Dickinson et al., 1999, 2000; Sane and Dickinson, 2001; Sane, 2003; Lehmann, 2004a,b; Thomas

et al., 2004; Balint and Dickinson, 2004; Sun, 2014). Though studying changes in wing pitch angle was outside of the scope of this study it certainly deserves further study, particularly as the gear change mechanism has been implicated in the control of this kinematic in *C. vicina* (Chapter 3).

The variations we have observed in other kinematics, such as stroke angle, deviation angle, stroke plane and wingbeat frequency cannot feasibly have been caused by the gear change mechanism with our current understanding of it. It is therefore more likely that the various control muscles that attach to the various components of the wing hinge are responsible for these changes in wing movements. There is a substantial amount of literature on these muscles, in terms of their anatomy, electrophysiology and biomechanics, all of which indicate their ability to influence the wing movements in the ways we have observed here (e.g. Nachtigall and Wilson (1967); Heide (1968, 1983); Egelhaaf (1989); Dickinson et al. (1993); Tu and Dickinson (1994); Lehmann and Götz (1996); Dickinson and Tu (1997); Fayyazuddin and Dickinson (1999); Balint and Dickinson (2004); Dickinson (2005); Walker et al. (2014); Lindsay et al. (2017)). *E. tenax* has never been studied in this respect, but the internal musculature of brachyceran dipteran insects is thought to be fairly conserved (Williams and Williams, 1943; Wisser and Nachtigall, 1984; Walker et al., 2014; Fabian et al., 2016), and so it is reasonable to assume that the same findings from species such as *C. vicina* and *Drosophila* would apply to this species too. *E. tenax* may therefore rely primarily on the control muscles to determine the majority of the wing’s kinematics.

4.4.2 The Gear Change Mechanism Between Species

Previous work has documented the varying anatomy of the *RS* and *PWP* across the Diptera (Miyan and Ewing, 1985a; Nalbach, 1989; Deora et al., 2015), finding that these two components vary significantly in both size and shape. We find in this study that this variation not only affects how many interactions the two components can engage in, but it may also affect the ability of this contact to restrict wing motion.

This in turn affects the ability of the gear change mechanism to influence wing movement during flight. The three gears of *C. vicina* results in three distinct sets of wing kinematics, in which *RS-PWP* interaction appears to restrict wing movement (Chapter 3). We have observed here that there are two sets of wing kinematics in *E. tenax*, associated with the two different types of *RS-PWP* interaction, but these are far less distinguishable than the three different gears exhibited by *C. vicina*. Figure 4.3 shows that the wing movements in which the *RS* and *PWP* are engaged (gears 1 and 3) display a more restricted anterior motion (a smaller maximum stroke angle), possibly caused by the interaction of the two components acting like a brake and inhibiting further movement. However, in *E. tenax*, the gear in which the two components make contact (gear 1) actually exhibits a greater maximum stroke angle than the gear in which they do not (gear 3). It therefore seems that *RS-PWP* contact may not inhibit wing movement in *E. tenax*.

If the *RS-PWP* does not control stroke angle in *E. tenax*, this further implies that the interaction does not inhibit wing motion in this species in the same way as is seen in *C. vicina* (Chapter 3). This is further corroborated by our finding that there is no significant difference between the mean wingbeat frequencies of between either gear of *E. tenax*. Conversely, we do see significant differences in the wingbeat frequencies across gears in *C. vicina*, with wingbeats that experience *RS-PWP* interaction showing higher mean wingbeat frequencies than those without the same interaction. This is important because the insect flight motor is thought to rely on resonance (Roeder, 1951; Greenewalt, 1960; Dickinson and Lighton, 1995; Brennan et al., 2003; Ha et al., 2013; Deora et al., 2017), and so an effective shortening of the wing length caused by the *RS-PWP* interaction (which effectively changes the wing’s pivot point about the thorax from the wing base to the *RS*) causes a reduction in the moment of inertia imposed on the system by the wing. As a result, the resonant frequency of the system increases (discussed in more detail in Chapter 3). As we do not observe the same thing in *E. tenax*, this implies there is significantly less inhibition of wing movement due to *RS-PWP* engagement.

Table 4.6: A table documenting the significance values of kinematic differences found associated with gear changing in both *C. vicina* and *E. tenax*. Arrows indicate the trend of the kinematic value from gear 3 to gear 1, and from a flat to a flipped alula. $\uparrow$ and $\downarrow$ denotes an increasing or decreasing value, respectively, while $\rightarrow$ and $\leftarrow$ denotes the end of the downstroke occurring later or earlier in the wingbeat. Bold values indicate results which are statistically significant after the application of a false discovery rate algorithm. φ = stroke angle (φ_{Range} therefore represents stroke amplitude), ϑ = deviation angle, ψ = stroke plane angle.

Kinematic	Effect of <i>RS-PWP</i> Engagement in:	
	<i>C. vicina</i>	<i>E. tenax</i>
φ_{Max}	$\downarrow - p < 0.001$	$\uparrow - p < 0.001$
φ_{Min}	$\downarrow$ (gear 2 to gear 1) $\uparrow$ (gear 3 to 1) $p < 0.001$	$\uparrow - p = 0.03$
φ_{Range}	$\downarrow - p < 0.001$	$\uparrow - p < 0.001$
ϑ_{Max}	$\uparrow - p < 0.001$	$\downarrow - p < 0.001$
ϑ_{Min}	$\downarrow$ (gear 2 to gear 1) $\uparrow$ (gear 3 to 1) $p < 0.001$	$\uparrow - p < 0.001$
ϑ_{Mean}	$\uparrow - p < 0.001$	$\downarrow - p < 0.001$
ϑ_{Range}	$\uparrow - p < 0.001$	$\downarrow - p < 0.001$
ψ_{Mean}	$\downarrow - p < 0.001$	$\downarrow - p < 0.001$
Relative Timing of Downstroke End (t/T)	$\rightarrow - p < 0.001$	$\leftarrow - p < 0.001$
Wingbeat Frequency (Hz)	$\uparrow - p < 0.001$	No significant difference $p = 0.885$

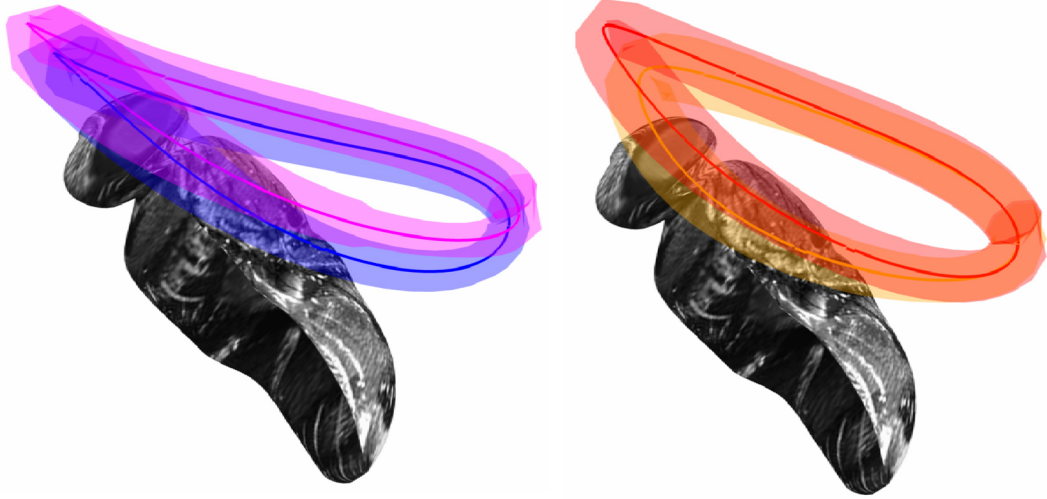


Figure 4.10: Three-dimensional wingtip paths taken during different gear-alula state combinations. The thin bold line represents the mean wingtip path, while the surrounding patch represents one standard deviation from this mean. Left: gear 1, blue - alula flipped ($n = 851$), magenta - alula flat ($n = 3262$). Right: gear 3, orange - alula flipped ($n = 600$), red - alula flat ($n = 845$).

The myriad of differences between these two species (table 4.6) may in part be explained by the variations in gear change mechanism anatomy. For example, in figure 4.2, the *PWP* of *C. vicina* is 149 μ m in height and 134.6 μ m in width, while that of *E. tenax* is only 92 μ m and 68 μ m in height and width, respectively, despite the wings of the *E. tenax* being much larger (around 10-12mm) than those of *C. vicina* (around 8-9mm). As a result, compared to *E. tenax*, the *PWP* of *C. vicina* is proportionally much larger relative to the wing, meaning that it may be more capable of inhibiting the wing's movement (assuming that the gear change mechanism does indeed act as a mechanical brake to influence wing kinematics). If this is indeed the case, the ability of the mechanism in *E. tenax* to inhibit movement would be comparatively limited, and it would only be capable of determining a smaller number of wing kinematics, such as wing rotation.

4.4.3 The Alula as a Gear Change Indicator

In a similar conclusion as that in our previous work on *C. vicina* (Chapter 3), we find here that the alula can occupy either a flat state or a flipped state regardless of the gear engaged. As a result, the alula cannot be used as a reliable indicator as

had been suggested as a possibility by (Walker et al., 2012). Despite this, we do find some relationship between the state of the alula and the gear engaged, such that a wingbeat in which the alula is flat is more likely to be engaged in a gear 1 than gear 3, while an alula-flipped wingbeat is more likely to be engaged in gear 3.

This relationship between the state of the alula and the gear engaged is similar to our previous findings in *C. vicina* (Chapter 3). Just as in *E. tenax*, this species displays a relationship in which certain alula states are more likely to be engaged in different gears (alula-flat: gear 2; alula-flipped: gears 1 or 3). That these correlations exist in both these species suggests there is some relationship between alula state and the gear engaged, though it is unlikely to be due to a common muscular mechanism which controls both these features.

Whatever the mechanism responsible for changing alula state is, it also appears to significantly alter wing kinematics (figure 4.10, tables 4.4 & 4.5). Alula-flat wingbeats, for example, show a greater maximum stroke angle in both gears 1 and 3, suggesting that these wingbeats are likely to move a greater volume of air than alula-flipped wingbeats, thereby producing a larger force. The smaller deviation range in alula-flat wingbeats will also mean that the wing is effectively beating with a smaller anhedral or dihedral angle, meaning that the resultant force vector is closer to being perpendicular to the sagittal plane of the body compared to the forces produced by alula-flipped wingbeats. Wingbeats such as these may therefore be utilised in situations where the insect must ascend vertically, for example, as they maximise the resultant force vector component which counteracts the weight of the insect. This effect will be further amplified by the increased area of the wing when the alula is in its flat position. These findings reinforce our conclusions in previous work (Chapter 3) that the alula functions to amplify or minimise aerodynamic force output from the wing, by moving to a flat or flipped position, respectively.

4.5 Conclusions

This study has documented the gear change mechanism for this first time in *E. tenax*, and determined the role of the mechanism in the control of wing kinematics. The two gears we see in *E. tenax* are associated with two distinct sets of wing movements, in the same way that the three possible gears in *C. vicina* are each associated with distinct types of wing movements. Unlike in *C. vicina*, however, where the *RS-PWP* interaction appears to influence wing movement through restricting wing motion, the interaction in *E. tenax* does not appear to be able to do this. Instead, we believe that the interaction aids in the control of stroke reversal, and possibly wing supination, at the end of the downstroke. These differences are likely influenced by the differences in the size of the *PWP* relative to the wing and body between these two species, with the *PWP* being both relatively and absolutely smaller in *E. tenax* compared to *C. vicina*.

We have also shown similar significant differences in wing kinematics depending on the state of the alula during the wingbeat. However, we have found no deterministic relationship between the state of the alula and the gear engaged, but we do find that certain alula states are more likely to be seen during different gear engagements. As a result, the muscular control underlying the determination of the gear change mechanism is still undetermined.

This study has revealed that the gear change mechanism varies significantly between species, and that this variation can provide further insight into its function. More comparative studies of this mechanism across species are warranted, as well as more detailed investigations into the biomechanics through which *RS-PWP* interaction acts to alter wing kinematics.

Chapter 5

The Biomechanics of the Gear Change Mechanism

Despite extensive investigations into the effects of *RS-PWP* interaction on wing kinematics in several species, the biomechanics and even the exact role of the gear change mechanism is still not clear. Here, we perform ablation experiments to determine the role of the mechanism, and quantify the movement of these components during tethered flight. We also study the motion of the wing before and after engagement, and during wingbeats transitioning between different gears to identify the impact of this interaction over the course of the wingbeat. We find that individuals are able to control their wing kinematics even with one of these components removed, and the engagement is determined by the motion of the wing at the start of each wingbeat. However, we also posit that when the two structures do interact in *Calliphora vicina*, the engagement absorbs kinetic energy from the wing, thereby altering movement. This kinetic energy absorption does not appear to occur in other species, however, and we thereby suggest that the gear change mechanism is a pivot about which kinematics such as wing rotation or amplitude can be controlled. Crucially, however, this interaction is not essential for the control of wing movements. Instead, it is likely to be an aid that improves the efficiency of the flight motor by reducing the amount of work performed by the control muscles, therefore aiding in

sustained flight performance.

5.1 Introduction

In Chapter 3 we hypothesised that the gear change mechanism in *Calliphora vicina* may act to control wing kinematics by inhibiting wing motion through the brake-like interaction of the *RS* and *PWP*. Wingbeats in which the *RS* engages with the *PWP* exhibit a reduced anterior motion of the wing at the end of the downstroke, which we theorise results from the *PWP* restricting further anterior motion of the *RS* (and the wing to which it is attached) when contact is made. However, the findings of the previous chapter studying the same mechanism in *Eristalis tenax* do not appear to conform to this theory. The kinematics of wingbeats in which the *RS* and *PWP* make contact show a greater extent of anterior motion at the end of the downstroke, and therefore seem unlikely to be produced if the interaction of the two sclerites acts to inhibit wing motion. Instead, we believe that, in *E. tenax* at least, the gear change mechanism may be responsible for controlling wing pitch, and aiding the reversal of the wing's motion at the end of the downstroke.

Both these hypotheses of the gear change mechanisms's function conform to previous theories posed by earlier authors. Nalbach, Miyan & Ewing, Ennos and Deora et al. all posit that the mechanism inhibits anterior wing motion (Miyan and Ewing, 1985a; Ennos, 1987; Wisser, 1988; Nalbach, 1989; Deora et al., 2015), while Pfau and Wisser assert that the mechanism is an aid for wing rotation (Pfau, 1973, 1987; Wisser, 1987). These theories may not be mutually exclusive, but it is important for our understanding of wing kinematic control that we draw conclusions regarding the function of the gear change mechanism that can be generalised across Diptera. To do this, a more detailed understanding of the role of each component, and their movements throughout the wingbeat, is required.

In this study, we have performed ablation experiments to observe the ability of insects to control their wing kinematics in the absence of a *PWP*, and have utilised high-speed macrophotography to examine the movements of the wing hinge

components in *C. vicina*. By tracking the motion of the *RS* and *PWP* throughout the wingbeat, we aim to identify changes in movement across different gears, and from this infer how the interaction influences their trajectories, and how the type of interaction is selected. Using this information regarding the time of interaction, we have also been able to document detailed changes in wing kinematics around this moment to highlight whether it has an immediate mechanical effect on wing motion. We have further explored this idea by utilising K-means clustering patterns of mean three-dimensional wingtip positions at several points during the wingbeat. This enables us to study variations in the movement of the wing throughout the wingbeat across different gears, so as to identify when the gear is determined and the effect of gear interaction on wing trajectories. Finally, by examining wingbeats that are transitioning across different gears and comparing them to those engaged in the same gear for several previous wing strokes, we hope to elucidate when the kinematic differences become apparent.

By studying the movement of the wing and gear change mechanism components, we make steps towards identifying when the gear of a wingbeat is determined. We find that, while the gear of the wingbeat appears to be determined before *RS-PWP* interaction, the engagement of the two components appears to alter the trajectory of the wing. By studying changes in stroke angle around the time and after engagement, we posit that this change results from a mechanical influence of the interaction on wing motion. However, the kinematics of transitional wingbeats reveal that this interaction is not sufficient to bring about the observed changes immediately, and that it requires several wingbeats engaged in the same gear continuously for the full changes to become apparent. Crucially, we also demonstrate that these insects are capable of controlling their wings with the same range of kinematics regardless of the presence of a *PWP*.

The results of this study therefore highlight a level of redundancy within this extremely complex system, and call into question the necessity of the gear change mechanism for wing kinematic control. We assert that *RS-PWP* interaction may

act to improve the efficacy and efficiency of wing kinematic control in Diptera by providing a pivot about which wing kinematics can be controlled and altered. In doing so, despite not necessarily being required in order to alter wing kinematics, the gear change mechanism may facilitate the sustained and precisely controlled bouts of flight that these insects demonstrate.

5.2 Materials and Methods

Unless otherwise specified, the methodologies used here relating to insect preparation, high speed camera set up, three-dimensional wing kinematic reconstruction and wingbeat gear classification, are all the same as those used in Chapter 3. In this section, any variations upon these methodologies or additional steps and techniques are explained.

5.2.1 Experimental Conditions

We observed bouts of flight under three experimental conditions: *PWP* intact (control), sham *PWP* removal, and *PWP* removed. In order to access the *PWP* for the removal and sham conditions, we cold anaesthetised the animals and placed the insect in a groove cut into a sponge. We then gently pressed the insect inside a small clamp, during which, the wings often raised and revealed the wing hinge for manipulation. If this did not happen, however, we restrained the wing in an upward position. Using two fine scalpel blades observed through a microscope, we then pinched the head of the *PWP* until it snapped off. Under sham conditions, we undertook the same steps, but only touched the *PWP* with the scalpels rather than removing it. We allowed the animals to rest for two hours after *PWP* manipulation, and then continued with the tethering and high-speed filming processes as carried out in Chapter 3. Unlike the conditions utilised in this previous chapter, however, the tethered flight conditions used here included a visual stimulus of a moving stripe pattern to help stimulate forward flight.

5.2.2 Two Dimensional Wing Hinge Component Tracking

Using the videos obtained from the camera filming the wing hinge we were able to track the movements of the *RS* and *PWP* throughout the wingbeat. After loading the video (filmed at 6,400 frames per second) into MATLAB, we clicked on the centre of both the *RS* and *PWP* individually once every four frames (providing adequate time resolution without the need to record every frame), giving ten measurements of both sclerites per wingbeat. We tracked the movement of these two components in nine videos from eight individuals of particular interest due to the variety of gears exhibited across the duration of the video.

We loaded the coordinates of the clicked points into a custom MATLAB script to split the data into different wingbeats. The script then normalised each dataset by the size of the *PWP* in the respective recording (measured at the distance between the anterior-most and posterior-most peaks on the *PWP* head), allowing the paths of both the *RS* and *PWP* to be plotted on the same axes. To quantify the features of these paths, we calculated the amplitude of component motion, which was defined as the maximum straight-line distance of the path from the point at the start of the wingbeat, and the displacement of the components along the horizontal and vertical axes of the video images, to determine antero-posterior and dorso-ventral movement respectively.

5.2.3 Transitional Wingbeat Classifications

To determine how influential *RS-PWP* interaction is in the determination of wing motion, we examined the kinematics of the wing during wingbeats that have transitioned from one gear to the next. For example, a gear 2 wingbeat that succeeds a gear 1 wingbeat would be classed here as a transitional wingbeat, and the measured kinematics are taken from the gear 2 wingbeat. However, a gear 2 wingbeat that succeeds another gear 2 wingbeat is classed as a continuous wingbeat.

To acquire a large enough dataset of transitional wingbeats, we made use of the larger set of wing kinematic data collected during Chapter 3. The experimental

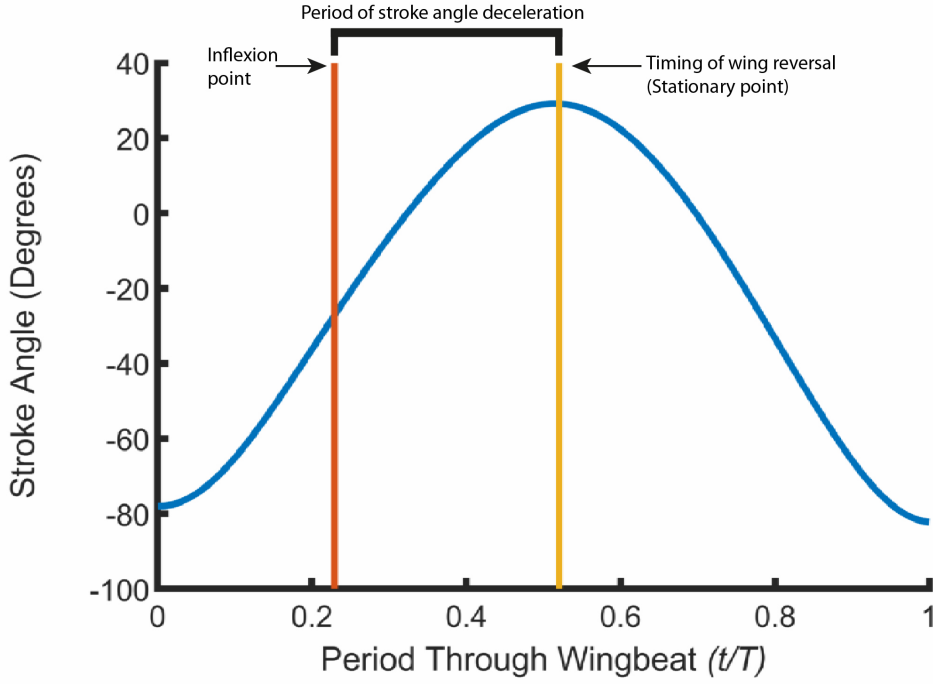


Figure 5.1: Stroke angle plotted through the wingbeat for one wingbeat (blue curve), illustrating the inflexion point (orange line) and the timing of stationary point (yellow line). The inflexion point shows the point at which the stroke angle is no longer accelerating, and the stationary point displays the time at which the stroke angle stops increasing. t is time, while T is the overall duration of the wingbeat, where t is equal to zero at the start of the downstroke.

conditions utilised in the gear change mechanism manipulation experiments included a striped pattern visual stimulus, meaning that the experimental conditions used in this previous chapter were not identical to those used here. Therefore, to avoid confounding results, these datasets have not been mixed, and the dataset from Chapter 3 has been used for this part of the study only.

5.2.4 K-means clustering of wingtip paths

Using MATLAB, we performed K-means clustering to group the three-dimensional wingtip positions (normalised by wing length) at different stages in the wingbeat. By examining the clustering patterns of these wingtip points, we can determine when during the wingbeat it was possible to distinguish different gears, if at all. For example, if the three-dimensional wingtip positions of gears 1, 2 and 3 are all allocated into three separate clusters, or show significantly different grouping patterns across the clusters, we can likely determine the gear of any given wingbeat

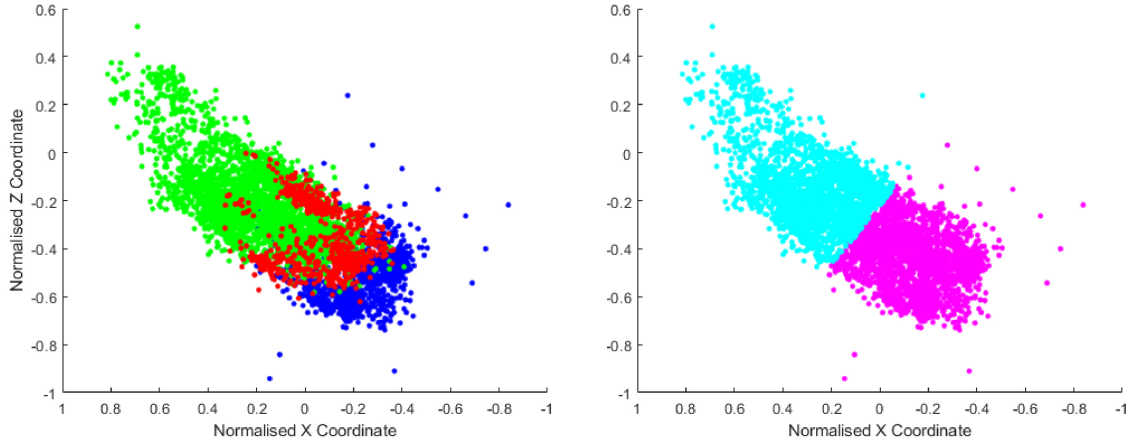


Figure 5.2: Mean two-dimensional projections of three-dimensional positions of the wingtips at the end of the downstroke (from $t/T = 0.4-0.6$) grouped into gears (left) and clusters (right) after the application of a 2-centroid K-means clustering algorithm (i.e. the algorithm which produces the greatest mean silhouette value). Blue - Continuous gear 1 ($n = 1359$); Red - Continuous gear 2 ($n = 709$); Green - Continuous gear 3 ($n = 3128$); Cyan - Cluster 1 ($n = 2412$); Magenta - Cluster 2 ($n = 2784$).

based on the wingtip position. We performed this k-means clustering on mean normalised three-dimensional wingtip positions before *RS-PWP* interaction ($t/T = 0 - 0.25$; t is time and T is the total duration of the wingbeat, where t is equal to zero at the start of the downstroke), and also on the average position around the end of the downstroke, when the *RS* and *PWP* were in contact ($t/T = 0.4 - 0.6$).

One other feature of the clusters, the mean silhouette value, measures the similarity of a point to other points in the same cluster by calculating the mean distance between each point to other points in its same cluster, and comparing this to the mean distance between each point to points in other clusters. The silhouette value therefore gives a measure of the distinctiveness of the clusters (Rousseeuw, 1987), and allows us to determine how easily distinguished different clusters are at different points in the wingbeat. If different gears cluster separately in k-means clustering, high silhouette values of these clusters indicates that the wingtip positions of differently geared wingbeats are easily distinguished. The greatest value a silhouette value a point can possess is 1, meaning that the point is very well matched to its cluster, whereas a value of -1 means that a point is poorly fitted to its cluster. By extension, a cluster with a mean silhouette value of close to 1 is a very distinct cluster, whereas a cluster with a mean silhouette value closer to -1 is not distinct

from neighbouring clusters.

In examining differences in these wingtip position cluster silhouette values taken across the two times in the wingbeat (across the first quarter of wingbeat, or first half of the downstroke, and the end of the downstroke), we can determine whether these clusters become more distinctive after the interaction of the *RS* and *PWP*. This methodology also allows us to determine how many clusters the data should be partitioned into to give the optimum silhouette value, and therefore identify how many clusters the data can be most readily grouped into. We therefore ran the K-means clustering algorithm with up to six centroids (creating the same number of clusters) for each set of pair of continuous gears and their respective transitional gears, and evaluated the mean silhouette value of these clusters to find the optimum number of clusters which generated the greatest value.

5.2.5 Rates of Stroke Angle Change

In another attempt to determine whether there is a mechanical effect on the wing as a result of *RS-PWP* contact, we observed whether the contact altered rates of stroke angle change during the downstroke. Using the plots of stroke angle across the wingbeat (figure 5.1) we calculated the point of inflexion, which indicated the point at which the stroke angle was no longer accelerating and begins to decelerate. We then calculated the rate of change between this point, and the point at which the stroke angle change is stationary at the end of the downstroke. Through ANOVA tests, we compared the timing of both the point of inflexion, and the rates of stroke angle deceleration, across gears 1, 2 and 3 to identify any impact of *RS-PWP* contact on the wing's movement.

5.2.6 Statistical Analysis

To determine statistically significant differences amongst the kinematics of wingbeats in different gears and experimental treatments, as well as between transitional wingbeats and continuously geared wingbeats, we used one-way analysis of vari-

ance (ANOVA) tests. Further to this, we utilised Tukey-Kramer tests to identify significant differences between pairs of gears or between treatments.

Finally, we applied the same false-discovery rate algorithm used in previous chapters across all our statistics here to minimise the risk of type I errors.

5.3 Results

5.3.1 Comparison of wing kinematics across experimental conditions

In total, we acquired twenty two control flights from eleven individuals (a total of 4,803 wingbeats), five sham-removal flights from three individuals (1,085 wingbeats), and fourteen *PWP*-removed flights from nine individuals (3,452 wingbeats). We found significant differences in each measured wing kinematic across each of the experimental conditions (Appendix B table B.1). Across the five sham recordings, the insects beat their wings exclusively in gear 3. Though the measured wing kinematics are significantly different to those of gear 3 wingbeats shown by control insects, the kinematics of these gear 3 wingbeats in sham individuals were still more similar to gear 3 wingbeats of control insects than those of any other gear (figure 5.3, table B.1). Given the large difference in sample size between the sham and control experiments (5 and 22 recordings from 3 and 11 individuals, respectively), these significant differences could stem from sampling artefacts, as the insects studied in the control tests were particularly strong fliers. Note that mean values of the kinematics measured in the sham *PWP* removal dataset all fall within the mean value of the gear 3 wingbeats of the control dataset plus or minus one standard deviation, which implies that these gear 3 wing kinematics of the sham dataset are not outside the plausible range of normal, control gear 3 wingbeats. We therefore conclude that, although the sham dataset shows differences to the control data, they do not do so in such a way as to assert that the experimental manipulation lead to the production wing kinematics that are far outside the normal range produced

during tethered flight, and the differences could stem from differences in sample size.

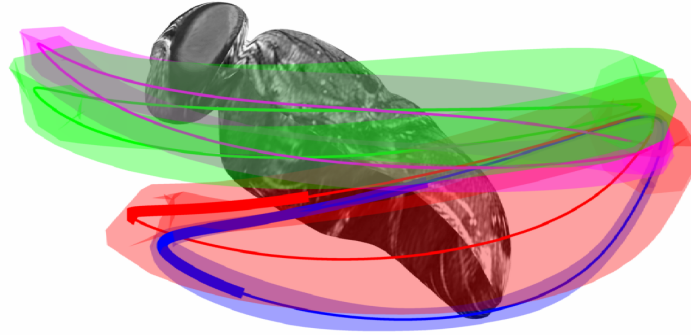


Figure 5.3: Mean paths of the wingtip during wingbeats across control and sham conditions. The thin solid line represents the mean path, while the transparent patch represents one standard deviation of this mean. The thicker blue and red lines illustrate the period of the wingbeat during which the *RS* and *PWP* are engaged in gears 1 and 2, respectively. Blue - Gear 1 Control ($n = 9$ wingbeats); Red - Gear 2 Control ($n = 254$); Green - Gear 3 Control ($n = 4540$); Magenta - Gear 3 Sham ($n = 1085$).

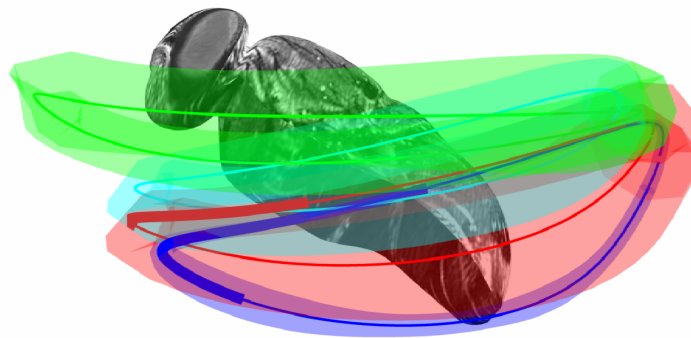


Figure 5.4: Mean paths of the wingtip during wingbeats across *PWP*-intact and *PWP*-removed experimental conditions. The thin solid line represents the mean of the paths, while the transparent patch represents one standard deviation of this mean. The thicker blue and red lines illustrate the period of the wingbeat during which the *RS* and *PWP* are engaged in gears 1 and 2, respectively. Blue - Gear 1 (Control, $n = 9$ wingbeats); Red - Gear 2 (Control, $n = 254$); Green - Gear 3 (Control, $n = 4540$); Cyan path - *PWP* Removed ($n = 3452$).

The wing kinematics recorded from the *PWP*-removed flights were also signif-

icantly different from every geared wingbeat in the control, *PWP*-intact dataset (table 5.1). This significance appears to stem from the kinematics being intermediate between the gears, being significantly different from each gear in at least one kinematic (figure 5.4). Crucially, the kinematics of *PWP*-removed wingbeats are significantly different to gear 3 in every measured wing kinematic, but are not significantly different to gears 1 and 2 in several cases. This suggests that the *PWP*-removed insects are capable of moving their wings with kinematics more similar to those of other gears, despite no *RS-PWP* interaction being possible (figure 5.5). From this, we conclude that *RS-PWP* contact is not essential for controlling wing kinematics, and that muscular control is capable of determining wing kinematics without making use of *RS-PWP* contact.

Table 5.1: Kinematics of the wings during control flights across different gears compared with PWP-removed wingbeats, presented as the mean $\pm$ one standard deviation and the resultant F - and p -values resulting from a one-way ANOVA test. The q -values resulting from Tukey-Kramer comparison tests between different gears and experimental treatments are also presented. Bold values indicate significant results after the application of a false discovery rate algorithm. φ = stroke angle (φ_{Range} therefore represents stroke amplitude), ϑ = deviation angle, α = wing pitch angle, ψ = stroke plane angle.

Kinematic	Treatment				Comparison with PWP Removed			F-Value
	Gear 1	Gear 2	Gear 3	PWP Removed	Gear 1	Gear 2	Gear 3	
φ_{Max}	23.1 $\pm$ 4	30.92 $\pm$ 13	54.23 $\pm$ 15	31.57 $\pm$ 15	$q_{6,3461} = 2.416$, $p = 0.319$	$q_{6,3706} = 0.954$, $p = 0.907$	$q_{6,7992} = 95.48$, $p < 0.001$	$F_{3,8251} = 1593$ $p < 0.001$
φ_{Min}	-79.99 $\pm$ 1	-80.15 $\pm$ 6	-78.29 $\pm$ 8	-74.98 $\pm$ 7	$q_{6,3461} = 2.738$, $p = 0.213$	$q_{6,3706} = 14.52$, $p < 0.001$	$q_{6,7992} = 26.74$, $p < 0.001$	$F_{3,8251} = 134.4$, $p < 0.001$
φ_{Range}	101.8 $\pm$ 5	108.4 $\pm$ 14	131 $\pm$ 15	104 $\pm$ 16	$q_{6,3461} = 0.7496$, $p = 0.952$	$q_{6,3706} = 5.434$, $p < 0.001$	$q_{6,7992} = 106.8$, $p < 0.001$	$F_{3,8251} = 1948$, $p < 0.001$
ϑ_{Max}	15.48 $\pm$ 2	12.18 $\pm$ 7	6.251 $\pm$ 8	10.4 $\pm$ 7	$q_{6,3461} = 2.719$, $p = 0.218$	$q_{6,3706} = 4.88$, $p = 0.003$	$q_{6,7992} = 32.8$, $p < 0.001$	$F_{3,8251} = 204.2$, $p < 0.001$
ϑ_{Min}	-7.37 $\pm$ 2	-7.741 $\pm$ 7	-16.53 $\pm$ 12	-9.764 $\pm$ 7	$q_{6,3461} = 1.016$, $p = 0.89$	$q_{6,3706} = 4.407$, $p = 0.01$	$q_{6,7992} = 42.45$, $p < 0.001$	$F_{3,8251} = 329.7$, $p < 0.001$
ϑ_{Mean}	0.7197 $\pm$ 0.9	0.3148 $\pm$ 3	-3.694 $\pm$ 7	-0.2303 $\pm$ 3	$q_{6,3461} = 0.7477$, $p = 0.952$	$q_{6,3706} = 2.203$, $p = 0.403$	$q_{6,7992} = 40.29$, $p < 0.001$	$F_{3,8251} = 289.2$, $p < 0.001$
ϑ_{Range}	22.85 $\pm$ 2	19.92 $\pm$ 8	22.78 $\pm$ 8	20.16 $\pm$ 9	$q_{6,3461} = 1.165$, $p = 0.843$	$q_{6,3706} = 0.5467$, $p = 0.98$	$q_{6,7992} = 16.77$, $p < 0.001$	$F_{3,8251} = 49.48$, $p < 0.001$
Mid-Upstroke α	114.6 $\pm$ 20	126.3 $\pm$ 18	131.8 $\pm$ 29	129.6 $\pm$ 22	$q_{6,3185} = 2.461$, $p = 0.303$	$q_{6,3415} = 2.728$, $p = 0.216$	$q_{6,7372} = 5.017$, $p = 0.002$	$F_{3,7616} = 7.77$, $p < 0.001$
Mid-Downstroke α	43.74 $\pm$ 39	51.6 $\pm$ 34	47.23 $\pm$ 21	35.86 $\pm$ 22	$q_{6,3185} = 1.535$, $p = 0.698$	$q_{6,3415} = 15.27$, $p < 0.001$	$q_{6,7372} = 31.45$, $p < 0.001$	$F_{3,7616} = 178.9$, $p < 0.001$
Relative Timing of Supination (t/T)	0.5764 $\pm$ 0.1	0.5344 $\pm$ 0.1	0.5071 $\pm$ 0.1	0.5882 $\pm$ 0.08	$q_{6,3196} = 0.5888$, $p = 0.976$	$q_{6,3441} = 14.53$, $p < 0.001$	$q_{6,7601} = 61.6$, $p < 0.001$	$F_{3,7658} = 633.4$, $p < 0.001$
Relative Timing of Downstroke End (t/T)	0.5699 $\pm$ 0.02	0.5503 $\pm$ 0.03	0.5169 $\pm$ 0.03	0.5297 $\pm$ 0.03	$q_{6,3461} = 5.195$, $p = 0.001$	$q_{6,3706} = 13.66$, $p < 0.001$	$q_{6,7992} = 24.62$, $p < 0.001$	$F_{3,8251} = 166.5$, $p < 0.001$
Wingbeat Frequency (Hz)	141.5 $\pm$ 3	139.7 $\pm$ 7	137.3 $\pm$ 13	160.2 $\pm$ 40	$q_{6,3461} = 2.874$, $p = 0.176$	$q_{6,3706} = 16.2$, $p < 0.001$	$q_{6,7992} = 52.06$, $p < 0.001$	$F_{3,8251} = 457.9$, $p < 0.001$

5.3.2 Wing kinematics across gear transition wingbeats

Moving on to study the kinematics of wingbeats transitioning from one gear to another, we collected a dataset of 7,054 wingbeats across 34 recordings of 15 individuals. Within these recordings, we observed every possible combination of gear transitions and a significant number of wingbeats which were engaged in the same gear for more than two wingbeats (i.e. continuous gear wingbeats). In total, we found 1,359 continuous gear 1 wingbeats, 709 continuous gear 2 wingbeats and 3,128 continuous gear 3 wingbeats. Furthermore, we observed 235 wingbeats transitioning from gear 1 to gear 2, 250 transitioning from gear 2 to 1, 147 wingbeats transitioning from gear 1 to 3, 136 transitioning from gear 3 to 1, 365 wingbeats transitioning from gear 2 to 3, and 373 wingbeats transitioning from gear 3 to gear 2. From these data, we were then able to identify kinematic differences between these transitional wingbeats, and their continuous-gear counterparts.

Here we have measured the kinematics of the latter wingbeat in the transition (i.e. the wingbeat after the gear change has occurred). For example in a gear 3 to gear 2 transition, we have measured the kinematics of the latter, gear 2 wingbeat. One-way ANOVA tests reveal that these transitional wingbeats exhibit significantly different wing kinematics compared to the continually-gear wingbeats that they are transitioning between (Appendix C). For example, a gear 2 wingbeat which has transitioned from a gear 3 wingbeat differs significantly when compared to continuous gear 2 and gear 3 wingbeats. This includes kinematics such as stroke amplitude and the timing of wing supination, which have been previously shown to be affected by *RS-PWP* interaction (Chapters 3 & 4; e.g. Pfau (1973); Nalbach (1989); Deora et al. (2015)). That the kinematics of transitional wingbeats were often intermediate between the continuously-gear wingbeats suggests that the gear change mechanisms takes effect over several wingbeats, and brings about changes in wing kinematics over this time period. The *RS-PWP* interaction is therefore not able to bring about the full differences we observe between different gears until at least two wingbeats have occurred in the same gear.

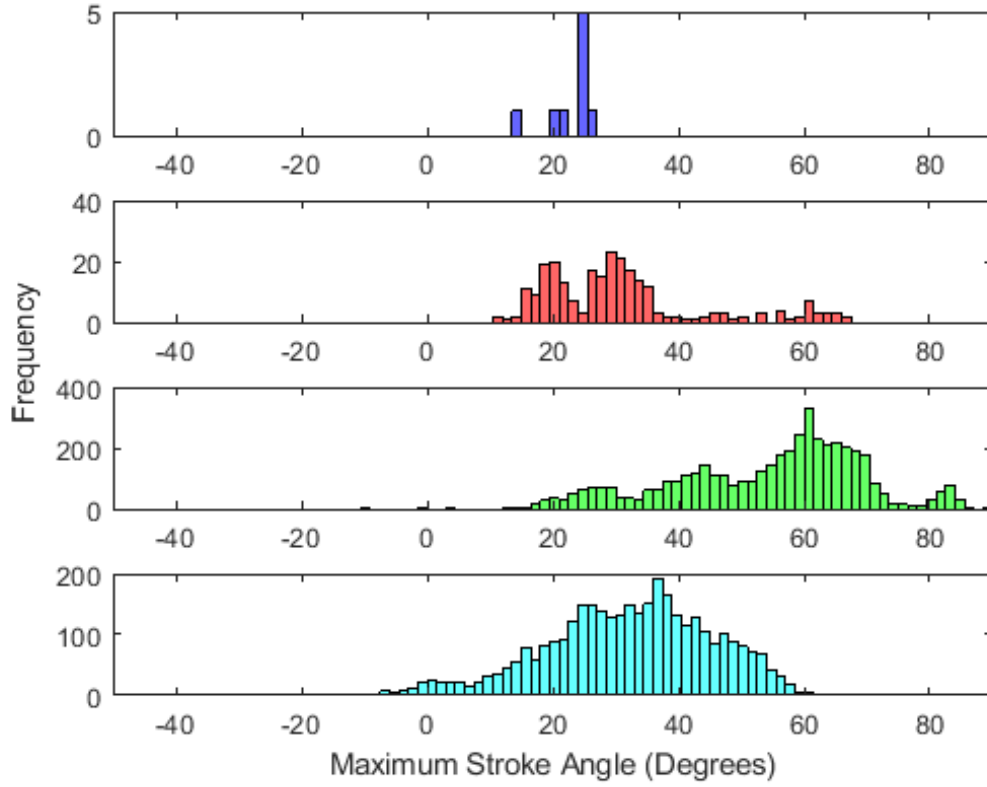


Figure 5.5: Histograms illustrating the maximum stroke angle of wingbeats in different gears and experimental conditions. Blue - gear 1 ($n = 9$); Red - gear 2 ($n = 254$); Green - gear 3 ($n = 4540$); Cyan - *PWP* Removed ($n = 3452$).

5.3.3 Distinction of wingtip paths across the wingbeat

Examining the trajectories of the wingtip during continuous wingbeats allows us to study the impact of *RS-PWP* interaction without the added variation in wingtip position introduced by transitional wingbeats. Grouping the mean wingtip positions of continuous-gear wingbeats over both the first half of the downstroke, and the end of the downstroke into different numbers of clusters reveals that the wingtip positions of the three different gears do not group into three distinctive clusters. The mean silhouette values of points in a two-cluster algorithm (0.6489 ± 0.3 and 0.6722 ± 0.2 , for the first quarter of the wingbeat and end of the downstroke, respectively) were significantly greater than those grouped in a three-cluster algorithm (0.5878 ± 0.2 , $t_{5195} = 14.15$, $p < 0.001$; and 0.5957 ± 0.2 , $t_{5195} = 16.78$, $p < 0.001$, for the first quarter and end of the downstroke, respectively). That the data more readily groups into two clusters rather than three suggests that there is significant overlap between

the wingtip positions across the three gears, rather than each of the gears producing wing kinematics with distinctive wingtip positions (tables 5.2 & 5.3, figure 5.2).

Further exploring the silhouette values produced by the more distinctive two-cluster algorithms (the algorithm producing the greatest mean silhouette score) reveal that the silhouette values differ significantly when calculated from mean wingtip positions across the first half of the wingbeat compared to wingtip positions from the end of the downstroke. The mean silhouette values of the clusters at the end of the downstroke are significantly greater (0.6722 ± 0.2) than those produced by the wingtip clusters during the first half of the downstroke (0.6489 ± 0.3 , $t_{5195} = 4.928$, $p < 0.001$), suggesting that the position of the wingtips are slightly more distinctive across gears at the end of the downstroke, after *RS-PWP* interaction, than they are before it.

Examining the z-values obtained from ransacking also reveals that, while the wingtip position clustering patterns of gear 1 wingbeats are not significantly distinctive from those of gears 2 or 3 at the beginning of the downstroke, they are distinctive at the end of the downstroke. As the position of the wingtip becomes more different and distinctive after *RS-PWP* interaction, this provides evidence for a likely role of the gear change mechanism in altering the path of the wingbeat. However, the wingtip positions of gears 2 and 3 cluster significantly differently even during the beginning of the downstroke, *before RS-PWP* interaction, which suggests that *RS-PWP* interaction may not be essential for changing the path of the wingtip at this stage. It is also interesting to note that the wingtip positions of gears 1 and 2 during the first half of the downstroke do not cluster significantly differently from each other, but do after *RS-PWP* interaction occurs at the end of the downstroke. This supports the idea that similar muscular mechanisms may determine whether the *RS* and *PWP* interact during the early stages of the wingbeat, but that the interaction (and the muscular mechanism that determines the type of interaction) is capable of changing wing kinematics further. By having two grooves (giving the two types of *RS-PWP* interaction) in the *PWP* head, *C. vicina* may be able to achieve

a greater resolution of wing kinematic control, compared with *Eristalis tenax*, for example, which possesses only 1 groove (Chapter 4).

Despite the clustering patterns of gear 2 wingbeat wingtip positions being significantly different from gear 3, it is hard to conclude with any certainty which gear a wingbeat is engaged in from the mean wingtip position across the first quarter of the wingbeat alone (table 5.2: a 55% chance of a gear 3 wingbeat and a 45% chance of a gear 1 or 2 wingbeat in cluster 1, or a 61% chance of a gear 3 wingbeat and 39% chance of a gear 1 or 2 wingbeat in cluster 2). However, from the clustering patterns of the differently geared wingbeats reported here (table 5.3), we can say with 66% certainty that a wingbeat with a mean wingtip position across the first quarter of the wingbeat located within cluster 1 is a gear 1 or 2 wingbeat, while we can say with 90% certainty that one located within cluster 2 is a gear 3 wingbeat.

Table 5.2: Clustering patterns of the mean wingtip position during continuous gear wingbeats across the first quarter of the wingbeat ($t/T = 0$ to $t/T = 0.25$). χ^2 value, p -value, adjusted residuals and z-values for the ransacking comparison tests are also given. Values in bold indicate statistically significant values.

<i>Continuous gears 1, 2 and 3 - 1st Quarter of Wingbeat</i>						
Gear				Adjusted Residuals		
	G1	G2	G3	G1	G2	G3
Cluster 1	284	182	580	0.82	3.958	-3.512
Cluster 2	1075	527	2548	-0.82	-3.958	3.512
χ^2 -Value	p -Value	Comparisons				
		G1 G2	G1 G3	G2 G3		
18.94	$p < 0.001$	2.268	1.261	3.528		

5.3.4 Action of *RS-PWP* interaction on wing movement across the wingbeat

After identifying that the position of the wingtip diverges more across gears after *RS-PWP* interaction compared to the position of the wingtip beforehand, we can study the movement of the wing around the moment of this contact to infer whether or not it has a mechanical influence on the wing's motion. There was a significant difference between the inflexion point of the stroke angle plots across the different gears ($F_{2,4394} = 8.802$, $p < 0.001$). Tukey-Kramer multiple comparison tests reveals that this significance stems from differences between the mean inflexion point time of gear 1 ($t/T = 35.66 \pm 8$) and gear 2 ($t/T = 27.43 \pm 9$; $q_{3,247} = 5.651$, $p < 0.001$) and gear 3 ($t/T = 27.22 \pm 6$; $q_{4159} = 5.896$, $p < 0.001$). The mean inflexion point timings of gears 2 and 3 were not significantly different ($q_{4388} = 0.7273$, $p = 0.864$). Despite our finding that the inflexion point (i.e. the time at which the rate of stroke angle change stops accelerating and begins decelerating) differs between gears 1 and 3, as we have not found a significant difference between those of gears 2 and 3, we cannot conclude that the *RS-PWP* interaction is responsible for these differences, and instead, subtleties in whatever mechanism is responsible for selecting between gears 1 and 2 may also cause the differences observed here.

Significant differences in the mean rate of stroke angle change were also present between the inflexion point and the stationary point across different gears ($F_{2,4394} = 357.9$, $p < 0.001$). Gear 3 showed the largest mean change in stroke angle per unit of time ($\frac{d\varphi}{dt} = 2.363 \pm 0.3$, mean $\pm$ one standard deviation, where φ is stroke angle, and t is time), which was significantly different from that of gear 2 ($\frac{d\varphi}{dt} = 1.853 \pm 0.3$; $q_{3,4159} = 36.24$, $p < 0.001$). Both these gears were in turn varied from gear 1 ($\frac{d\varphi}{dt} = 1.568 \pm 0.2$; $q_{3,247} = 3.975$, $p = 0.014$; $q_{3,4159} = 11.28$, $p < 0.001$; compared against gears 2 and 3, respectively). The smaller change in stroke angle over time in gears 1 and 2 suggests that stroke angle is not increasing towards maximum stroke angle at as great a rate as in gear 3. This further implies that the wing decelerates from the inflexion point to the stationary point significantly faster in gears 1 and 2

Table 5.3: Clustering patterns of the mean wingtip position during continuous gear wingbeats around the end of the downstroke ($t/T = 0.4$ to $t/T = 0.6$). χ^2 value, p -value, adjusted residuals and z-values for the ransacking comparison tests are also given. Values in bold indicate statistically significant values.

<i>Continuous gears 1, 2 and 3 - End of Downstroke</i>						
Gear				Adjusted Residuals		
	G1	G2	G3	G1	G2	G3
Cluster 1	64	154	2194	-35.88	-14.19	42.17
Cluster 2	1295	555	934	35.88	14.19	-42.17
χ^2 -Value		p -Value		Comparisons		
				G1	G1	G2
				G2	G3	G3
1832		$p < 0.001$		10.66	23.85	13.19

compared to gear 3. *RS-PWP* interaction is therefore correlated with decelerating the wing towards the end of the downstroke, even if it is not involved with beginning this deceleration in the first place.

5.3.5 Paths of the *RS* and *PWP* across the wingbeat in different gears

We further studied the possible restriction of wing motion by the gear change mechanism by studying the detailed motion of the individual components throughout the wingbeat. The path of both the *RS* and *PWP* differs across wingbeats in different gears (figure 5.6). In the movement of the *RS*, there is a significant difference in the amplitude of motion (distance from its position at the start of the wingbeat) across each gear ($F_{2,2175} = 704.5$, $p < 0.001$), with post-hoc multiple comparison tests revealing that mean amplitude of motion increases significantly from gear 1 (2.053 ± 0.017 normalised units of distance, mean $\pm$ one standard deviation) to gear 2 (2.396 ± 0.023 units of distance, $p < 0.001$), and from gear 2 to gear 3 ($2.971 \pm$

0.018 units of distance, $p < 0.001$). The amplitude of *PWP* movement also differs significantly across gears ($F_{2,2175} = 134.8$, $p < 0.001$), but multiple comparison tests show no significant difference between the mean of gear 1 (0.890 ± 0.007 units of distance) and gear 2 (0.887 ± 0.009 units of distance, $p = 0.971$), but that gear 3 movement amplitude (1.043 ± 0.008 units of distance) was significantly greater than both gear 1 and gear 2 ($p < 0.001$).

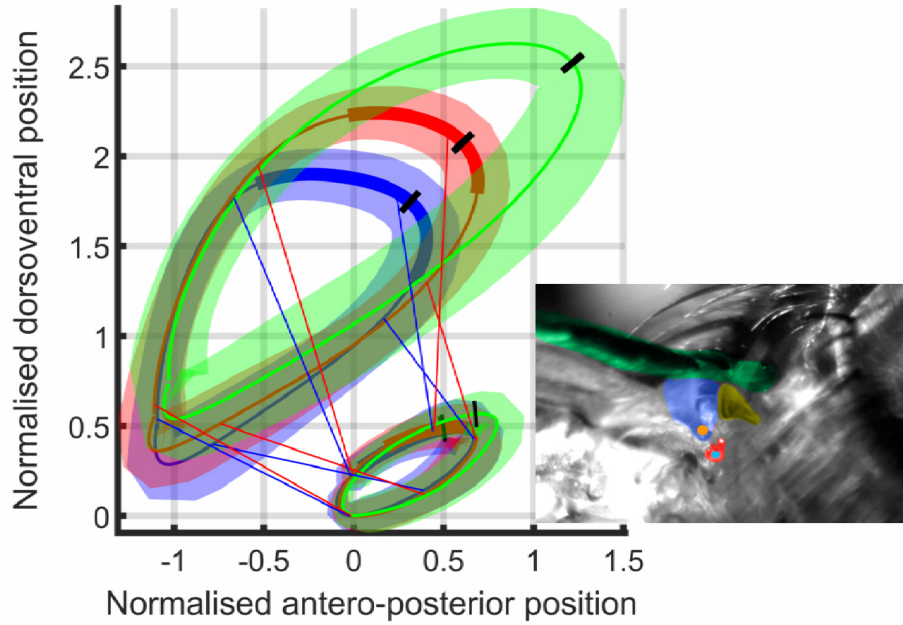


Figure 5.6: Two-dimensional paths of the radial stop (larger, top left) and pleural wing process (smaller, bottom right) during wingbeats in different gears, normalised by the width of the *PWP*. The thin bold line shows the mean path, while the wider translucent patch shows one standard deviation of this mean. The thickened bold line represents the period over which the *RS* and *PWP* are engaged. The black line indicates the mean time of wing supination during each gear. The coloured lines connect the *RS* and *PWP* paths at the same point in the wingbeat. The amplitude of both components is the straight line distance between the left-most and right-most points in the paths. Blue - Gear 1 ($n = 816$ wingbeats); Red - Gear 2 ($n = 504$); Green - Gear 3 ($n = 858$). The panel on the right shows a zoomed in image of a wing hinge, with the *RS* highlighted in blue and the *PWP* in red. The points tracked on both the *RS* and *PWP* during video analysis are shown in orange and light blue, respectively.

5.3.6 Paths of the *RS* and *PWP* relative to the wing

Finally, we combined this information regarding the variation in both the *RS* and *PWP* motion across gears and correlated it with the differences we have observed in wing motion. This enabled us to determine whether the changes in the movement of

these components directly influence wing kinematics, particularly how the restricted anterior motion of the *RS* (due to *PWP* contact) dictates the path of the wing to which it is attached. ANOVA tests reveal that the time lag between the *RS* reaching its greatest displacement along the x-axis (a measure of antero-posterior movement), and the time of the maximum stroke angle of the wing differs significantly across gears ($F_{2,2174} = 71.623$, $p < 0.001$). Tukey-Kramer multiple comparison tests further show that this mean time lag is greatest during gear 1 wingbeats ($t/T = -7.849 \pm 0.153$), and the mean magnitude of this lag decreases significantly through to gear 2 ($t/T = -6.986 \pm 0.195$, $q_{3,1320} = 4.935$, $p = 0.001$), and from gear 2 to gear 3 ($t/T = -5.328 \pm 0.149$, $q_{3,1362} = 9.568$, $p < 0.001$).

Furthermore, there is also a difference between the stroke angle of the wing at the maximum x-axis displacement of the *RS* and the maximum stroke angle of the wing during that wingbeat (figure 5.7). This difference differs significantly across gears ($F_{2,2174} = 28.364$, $p < 0.001$). Again, the mean difference is greatest in gear 1 ($-7.715 \pm 0.435^\circ$), decreasing significantly to gear 2 wingbeats ($-5.334 \pm 0.555^\circ$, $q_{3,1320} = 4.78$, $p = 0.002$), and again between gear 2 and gear 3 wingbeats ($3.136 \pm 0.424^\circ$, $q_{3,1362} = 4.543$, $p < 0.001$).

Together, these results show that the wing's inertia causes it to undergo slight span-wise bending throughout the wingbeat, meaning that the stroke angle of the wing at its base is slightly smaller than the angle at the wingtip. The fact that this difference is greater during wingbeats where there is *RS-PWP* interaction further implies that the wing's movement at its base is restricted by the mechanical interaction. This restriction means that the wing's inertia causes it to bend to a greater extent. In turn, this would explain the greater difference in magnitude and timing of maximum stroke angle between the wing base and wing tip during gear 1 and 2 wingbeats compared to wingbeats in gear 3.

5.4 Discussion

5.4.1 Effects of *PWP* removal on wing kinematics

There are several theories regarding how *RS-PWP* interaction works to influence wing kinematics, and indeed how vital it is, if at all, for the control of the wings in dipteran insects. The results of our ablation tests here demonstrate that insects are capable of showing the same range of wing kinematics regardless of the presence of a *PWP*, and the absence of the gear change mechanism as a result. During experimentation we also found that *PWP*-ablated insects released within the lab were able to control their flight without any noticeable impediment. Furthermore, our analysis of the wing kinematics of insects with and without the *PWP* intact (figure 5.4, table 5.1) show that flies without an intact *PWP* are not restricted to wing kinematics found in gear 3 wingbeats (where no *RS-PWP* interaction would normally occur). Instead, the mean wing kinematics from these insects were intermediate between gears, in many cases being significantly different from gear 3 wingbeat kinematics, and being more similar to gear 2 or gear 1 kinematics, implying that they are able to produce wing kinematics similar to those gears despite the absence of a *PWP*.

Previous authors have suggested that the *RS-PWP* interaction is crucial for the production or control of kinematics such as stroke amplitude or wing supination (Ennos, 1987; Wisser, 1988; Nalbach, 1989; Wisser and Nachtigall, 1991; Deora et al., 2015). However, our results show that *RS-PWP* interaction is not essential for producing any value of the wing kinematics studied here. This implies that there are muscular mechanisms capable of determining wing kinematics without the requirement of any mechanical interaction between the two sclerites.

That wing kinematics can be controlled independently of the gear change mechanism suggests that it is somewhat redundant within the flight motor. However, given the variety of forms the *RS* and *PWP* take across Diptera (Miyan and Ewing, 1984), it is difficult to believe that *RS-PWP* interaction is not important in some way. It may be the case that the mechanical interaction between the two

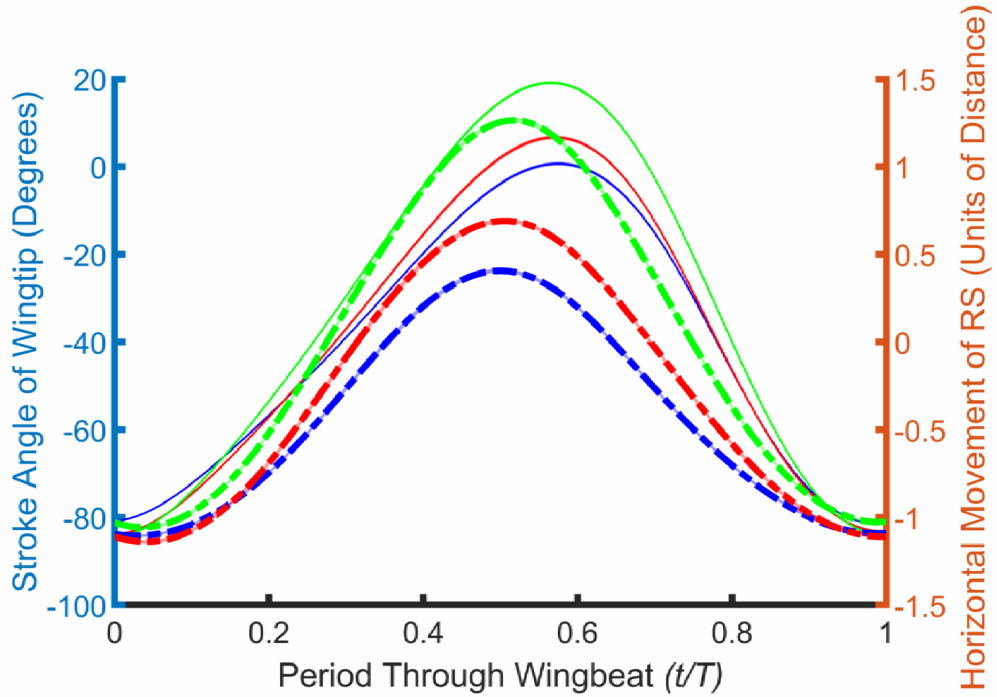


Figure 5.7: Stroke angle at the wingtip over the course of the wing beat (left y-axis) plotted with the horizontal movement of the radial stop (right y-axis). Solid lines - wingtip stroke angle; Dashed lines - *RS* movement. Blue Lines - Gear 1 wingbeats ($n = 816$); Red lines - Gear 2 wingbeats ($n = 504$); Green lines - Gear 3 wingbeats ($n = 858$).

components modified wing movement in a manner that would otherwise be done by the work of the control muscles, explaining why wing kinematics can be controlled even in insects where the *PWP* has been experimentally removed. The gear change mechanism may therefore be an energy saving mechanism, and a way of producing sets of wing kinematics that are frequently used during flight. The differences seen among species may be the result of requirements for certain sets of wing movements in the various lifestyles and ecologies in which these species live.

It would be interesting to observe the flight performances of insects with both *PWPs* ablated, and compare this to control individuals to determine whether the manoeuvrability or endurance of experimental individuals is compromised. However, these experiments are outside the scope of this study.

5.4.2 Determination of gears

Throughout investigations into the gear change mechanism, several attempts have been made to explain how different gears are selected (Pfau, 1973; Wisser, 1988; Nalbach, 1989; Balint and Dickinson, 2001; Walker et al., 2012; Deora et al., 2015, 2017). Some authors claim that some indirect flight muscles, namely the pleurosternal and tergopleural muscles, are capable of moving the *PWP* (Miyan and Ewing, 1985a; Pfau, 1987). They claim that activity in these muscles can cause medio-lateral movement of the *PWP* head, thereby placing the *PWP* in the path of the *RS* or allowing the two to bypass each other. Other authors, however, instead believe that the movement of the wing determines the gear. These authors claim that muscles of the first or third axillary sclerites, or the basalar sclerite, are responsible for causing the wings to move in a path where the *RS* and *PWP* make contact (Miyan and Ewing, 1985a; Nalbach, 1989; Balint and Dickinson, 2001; Walker et al., 2012).

Our attempts to group the mean three-dimensional positions of the wingtip during the first quarter of the wingbeat into different clusters reveal that wingtip positions of wingbeats in gear 1 do not significantly differ from either gears 2 or 3. However, mean wingtip positions of gear 2 show significantly different clustering patterns compared to gear 3 (though not gear 1), despite the *RS* and *PWP* not having been in contact by this point. This finding suggests that, at least in gear 2, the movement of the wing that separates it from the path of gear 3 wingbeats must be determined by the path of the wing *before RS-PWP* engagement, and not *by* the engagement itself. Further data may clarify whether this finding can also be extended to other gears, or whether our findings here hold true with a larger dataset. It should be stressed that, because none of these gears are contained exclusively within one cluster, it is not possible to definitively determine the gear of a wingbeat based on the position of the wingtip, and it is only possible to identify the probabilities of a certain gear being engaged. For example, there is a 45% chance of the wingbeat being engaged in gears 1 or 2 if the mean wingtip position over the first quarter of the wingbeat is grouped into cluster 1 (and a 55% chance of it being

a gear 3 wingbeat; table 5.2), and conversely there is a 39% chance of the wingbeat being engaged in gears 1 or 2 when the wingtip position is found in cluster 2.

These findings suggest that gear selection may occur before *RS-PWP* engagement, and as a result we believe that differential movement of the *PWP* is unlikely to be involved in the determination of gears. Though we did find variation in the movement of the *PWP* across different gears, we believe that these are due to differential deformations in the surrounding thorax corresponding to differences in wing movement such as stroke angle. It appears that tilting the stroke plane nose-down may cause the wing to move in such a path that the *RS* catches on one of the grooves of the *PWP*, leading to the engagement of the two components. The muscular mechanisms that determine the stroke plane may therefore be responsible for whether or not these components interact, but it should be noted that there is still substantial overlap between the recorded stroke planes of different gears (table 5.1), so there must be other factors involved in determining gear engagement. These findings lend support to the theories of Walker et al. (Walker et al., 2012), who suggest that the muscles of the third axillary sclerite determine gear engagement, or the muscles of the basalar sclerite as suggested by Nalbach (Nalbach, 1989). Whether these mechanisms are precise enough to control which groove of the *PWP* the *RS* catches on in *C. vicina*, or if multiple grooves simply increase the likelihood of interaction over a wider range of wing movements, as has been suggested by Deora et al. (Deora et al., 2017), is still a matter for debate.

Informed conjecture on the precise mechanism is outside the scope of this study, but it has provided a set of wing kinematics which are indicative of each gear. Modern methods such as time-resolved synchrotron X-ray computed microtomography (Walker et al., 2014), real-time calcium imaging (Lindsay et al., 2017) or electrophysiology will enable us to determine muscular movement and activity during wingbeats characteristic of different gears. This will then allow us to determine which muscles are responsible for gear selection, and the mechanisms through which this is achieved.

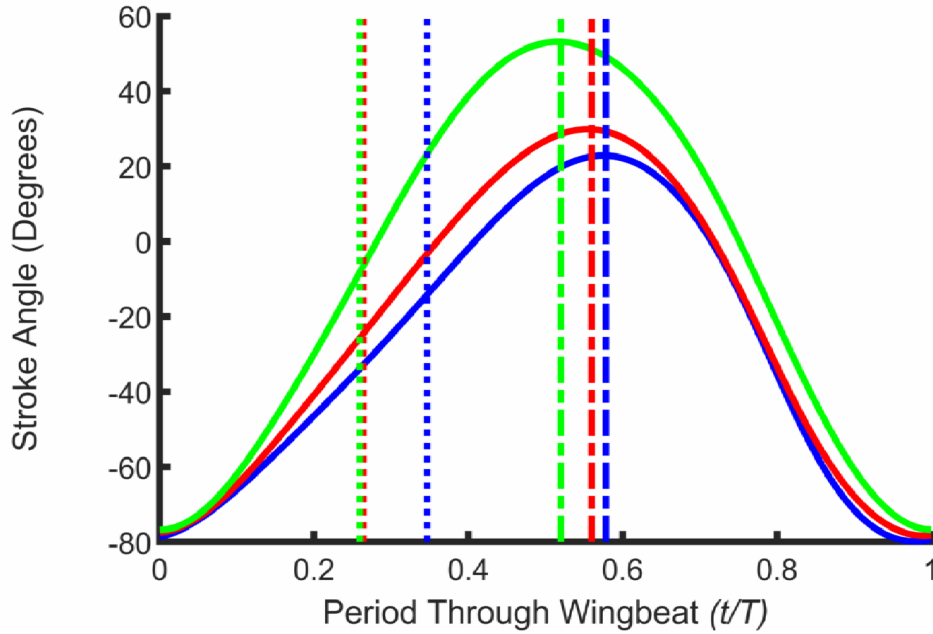


Figure 5.8: Stroke angle plotted across the wingbeat, indicating the graph's inflexion points (dotted lines) and the timings of stroke reversal as judged by the graph's stationary points (dash dotted lines). Green - gear 3; Red - gear 2; Blue - gear 1.

5.4.3 The mechanics of the gear change mechanism

That the *PWP* does not select the gear of the wingbeat does not preclude its involvement in determining wing kinematics. We and other authors have put forward theories to explain how the interaction brings about the observed kinematic changes. These range from the mechanical interaction acting as a brake-like mechanism to restrict wing movement (Chapters 3 & 4; Miyan and Ewing (1985a); Ennos (1987); Nalbach (1989); Wisser (1988)), to a pivot aiding wing rotation about its long axis (Pfau, 1973, 1987; Wisser, 1987).

Tracking the movement of the *RS* and *PWP* across differently geared wingbeats reveals that while the movement of the *PWP* does not differ significantly between gears 1 and 2, the movement of the *RS* does (figure 5.6). Given that the *PWP* position changes very little at the point of *RS-PWP* engagement between gears 1 and 2, it is reasonable to assume that the differences in *RS* movement are responsible for determining the gear engaged. This lends weight to the idea that contact between the two components determines the movement of the *RS*, and, by extension, will

determine the movement of the wing to which it is attached. The increased anterior motion of the *PWP* we observe in gear 3 wingbeats is likely a corollary of the entire wing hinge area being drawn further anteriorly by the movement of the wing and the surrounding thorax deforming to a greater degree.

Our data presented here also show that the wing decelerates towards this maximum stroke angle significantly differently across different gears (figure 5.8). Wingbeats in gears 1 and 2 showed significantly faster deceleration towards the end of the downstroke compared to gear 3 wingbeats. There was even a significant difference between gears 1 and 2, with gear 1 decelerating faster than gear 2. Despite the fact that this deceleration begins earlier than the time of *RS-PWP* engagement during these wingbeats, this still leaves open the possibility of the interaction influencing the motion of the wing during the downstroke along with the various muscular mechanisms and aerodynamic influences. This information supports the theory that *RS-PWP* interaction acts as some form of mechanical brake on the wing in *C. vicina*: the contact may absorb kinetic energy from the wing, which explains the changes in the rate of wing deceleration between the gears.

A final piece of support for the *RS-PWP* brake theory in this species comes from our finding that there are discrepancies between the maximum anterior movement of the *RS* and that of the wingtip. As well as this, there are significant differences in the magnitude of these discrepancies across the gears (figure 5.7). We have also found a delay in the time at which this maximum anterior movement is reached between the *RS* and wingtip. This suggests that at the end of the downstroke the inertia of the wing carries the tip farther anteriorly than the base, likely because the momentum of the wing increases along its length which in turn leads to some span-wise bending moments. During gears 1 and 2, the anterior movement of the *RS* is halted earlier in the wingbeat, but the wing likely has a similar amount of momentum as it would do during a gear 3 wingbeat. This results in a greater discrepancy in the maximum anterior movements of the *RS* at the wing's base and the wingtip, and a greater time delay too.

That the wing's inertia has a significant effect on its movement is suggested by our finding that kinematics of wingbeats transitioning between different gears are significantly different to those of wingbeats which have consistently been in the same gears they are transitioning between (see tables in Appendix C). For example, wingbeats transitioning from gear 3 on the previous wingbeat to gear 2 during the current wingbeat exhibit kinematics which are intermediate between the kinematics of wingbeats which have been consistently engaged in gears 2 and 3 (i.e. both the current and the previous wingbeat have been engaged in the same gear), and are significantly different to both of them. This suggests that the kinematics of the wing cannot be changed by *RS-PWP* interaction (or lack thereof) over the duration of one wingbeat due to the wing's inertia making it resistant to large changes in motion. As a result, the variation we observe between different gears (figure 5.4) must require at least two wingbeats engaged in the same gear to be fully realised.

We suggest that the gear change mechanism in *C. vicina* restricts wing movement by absorbing some of the its kinetic energy, but observations of tethered flight in *Eristalis tenax*, however, do not appear to conform to this theory. In this species, wingbeats in which the *RS* and *PWP* engage show a greater stroke amplitude than wingbeats in which there is no interaction (Chapter 4), and so it is not possible for the gear change mechanism to control wing kinematics by restricting wing movement. Instead, the interaction may help to control the timing of the end of the downstroke, and possibly aid wing rotation by providing a pivot about which the wing can rotate. The control of wing rotation by the gear change mechanism is also supported by our findings in *C. vicina* here and in previous studies (Chapter 3). We therefore believe that the gear change mechanism acts as a pivot about which the wing can rotate in both species, but in some species such as *C. vicina* where the *PWP* is large (figure 4.2), this interaction is also capable of inhibiting wing motion.

Pfau's competing theory (Pfau, 1973, 1987) that the *RS* and *PWP* only contact on the upstroke and may aid in wing pronation is not supported by the data presented here. Though we have previously observed a very rare interaction as part of

previous studies (fewer than 0.1% of the recorded wingbeats in Chapter 3), we believe that these wingbeats were extreme kinematics introduced by the tethered flight conditions as the kinematics of the wings during such wingbeats were not conducive to aerodynamic force production (as stroke amplitude was much reduced compared to normal bouts of sustained tethered flight). Indeed, the methods utilised by Pfau have been criticised previously, and so we believe that this author's findings may have been confounded by experimental errors relating to the use of CCl_4 analgesic on the insects (Miyan and Ewing, 1985a; Ennos, 1987).

Another theory put forward by Deora et al. (Deora et al., 2017) is that the *PWP* is only a frictional rest upon which the *RS* can lie during amplitude modulation. They also extend this to say that the two grooves on the *PWP* in *Calliphora* act only to improve this friction, rather than providing distinct gears. Our results here show that gear 1 and gear 2 wing movements have distinct clustering patterns during the latter half of the downstroke after *RS-PWP* engagement (table 5.3), and we see significant discrepancies between the maximum anterior movements of the radial stop and the wingtip across these two gears (and the delay in the time at which these points are reached; figure 5.7). These results strongly suggest that the *RS-PWP* does inhibit wing motion, and may not simply be a frictional stop in *C. vicina*, but it is unclear whether the two grooves are utilised separately or whether they increase friction and enable engagement to occur over a greater range of wing kinematics. Evidence from *E. tenax* does indeed suggest that the interaction, in this species at least, does not inhibit wing motion and may instead be a frictional pivot about which the wing can rotate and other wing kinematics can be altered.

A consistent argument many authors pose against the downstroke interaction of these two components is the requirement of the two to interact with such precision at such high speed and without damage to either part (Pfau, 1987; Deora et al., 2017). However, where Ennos, Miyan and Ewing suggest the *RS* and *PWP* contact on almost every wingbeat (Miyan and Ewing, 1985a; Ennos, 1987), we reaffirm findings by Nalbach and Deora et al (Nalbach, 1989; Deora et al., 2015) that the

two do not always interact (i.e. gear 3 wingbeats), which will reduce the amount of potential wear.

Our videos of the wing hinge during tethered flight show that this interaction does indeed occur with precision and at high speed, and can change on a wingbeat-by-wingbeat basis. This interaction may be aided by the fact that the *RS* can sometimes be seen to press against the lateral side of the *PWP* head before locking into one of the two grooves, with the *PWP* grooves therefore acting as a catch for the *RS*. Nevertheless, given the small size of the *PWP* compared to the available paths of the wing, some degree of precision is still required. The scepticism of previous authors regarding this ability is understandable, but our study confirms its occurrence, and the mechanisms underlying this feat deserve further study.

5.5 Conclusions

The gear change mechanism appears to be readily utilised by dipteran insects to help control wing kinematics. We suggest here that the interaction is a pivot about which wing kinematics can be altered, either by acting as a mechanical brake, absorbing kinetic energy and altering wing kinematics, or aiding in wing pitching. Crucially, however, we have determined that the interaction is not essential for wing kinematic control, as insects without the capability to utilise the gear change mechanism are capable of controlling their wing kinematics in a similar manner to unmanipulated individuals. As well as this, our results show that the path of the wing is determined before the *RS* and *PWP* interact, so gear selection likely takes place independently of the *PWP*, and muscular activity determining the path of the *RS* are responsible for determining the gear of the wingbeat. We thereby suggest that the gear change mechanism is an energy saving mechanism, which is used to alter wing kinematics without the need for the control muscles to perform work. The findings of this study illustrate how dipteran insects may have achieved such a remarkable combination of efficiency and precision in wing kinematic control. Detailed biomechanical studies such as this one are required for us to understand extremely complex systems like

the dipteran flight motor, and can in turn be used to inform the design of novel, more effective oscillating mechanisms such as those within flapping wing vehicles.

Appendices

Appendix B

Wing Kinematic ANOVA Results

Table B.1: Kinematics of the wings during control flights across different gears compared with *PWP*-removed wingbeats, presented as the mean $\pm$ one standard deviation and the resultant *F*- and *p*-values resulting from a one-way ANOVA test. The *q*-values resulting from Tukey-Kramer comparison tests between different gears and experimental treatments are also presented. Bold values indicate significant results after the application of a false discovery rate algorithm. φ = stroke angle (φ_{Range} therefore represents stroke amplitude), ϑ = deviation angle, α = wing pitch angle, ψ = stroke plane angle, t = time and T = the duration of the whole wingbeat.

Kinematic	Treatment				Comparison with Sham Gear 3			F-Value
	Gear 1	Gear 2	Gear 3	Sham Gear 3	Gear 1	Gear 2	Gear 3	
φ_{Max}	23.1 $\pm$ 4	30.92 $\pm$ 13	54.23 $\pm$ 15	53.62 $\pm$ 7	$q_{6,891} = 9.114$, $p < 0.001$	$q_{6,1136} = 31.89$, $p < 0.001$	$q_{6,5422} = 1.67$, $p = 0.639$	$F_{3,5681} = 232.1$, $p < 0.001$
φ_{Min}	-79.99 $\pm$ 1	-80.15 $\pm$ 6	-78.29 $\pm$ 8	-86.75 $\pm$ 10	$q_{6,891} = 3.392$, $p = 0.077$	$q_{6,1136} = 15.57$, $p < 0.001$	$q_{6,5422} = 38.63$, $p < 0.001$	$F_{3,5681} = 249$, $p < 0.001$
φ_{Range}	101.8 $\pm$ 5	108.4 $\pm$ 14	131 $\pm$ 15	139.2 $\pm$ 8	$q_{6,891} = 11.01$, $p < 0.001$	$q_{6,1136} = 42.64$, $p < 0.001$	$q_{6,5422} = 22.08$, $p < 0.001$	$F_{3,5681} = 318.4$, $p < 0.001$
ϑ_{Max}	15.48 $\pm$ 2	12.18 $\pm$ 7	6.251 $\pm$ 8	3.21 $\pm$ 5	$q_{6,891} = 6.517$, $p < 0.001$	$q_{6,1136} = 22.4$, $p < 0.001$	$q_{6,5422} = 14.7$, $p < 0.001$	$F_{3,5681} = 93.01$, $p < 0.001$
ϑ_{Min}	-7.37 $\pm$ 2	-7.741 $\pm$ 7	-16.53 $\pm$ 12	-14.58 $\pm$ 7	$q_{6,891} = 2.764$, $p = 0.206$	$q_{6,1136} = 12.34$, $p < 0.001$	$q_{6,5422} = 6.805$, $p < 0.001$	$F_{3,5681} = 57.36$, $p < 0.001$
ϑ_{Mean}	0.7197 $\pm$ 0.9	0.3148 $\pm$ 3	-3.694 $\pm$ 7	-4.042 $\pm$ 4	$q_{6,891} = 3.3$, $p = 0.091$	$q_{6,1136} = 14.2$, $p < 0.001$	$q_{6,5422} = 2.201$, $p = 0.404$	$F_{3,5681} = 38.3$, $p < 0.001$
ϑ_{Range}	22.85 $\pm$ 2	19.92 $\pm$ 8	22.78 $\pm$ 8	17.79 $\pm$ 7	$q_{6,891} = 2.174$, $p = 0.415$	$q_{6,1136} = 4.295$, $p = 0.013$	$q_{6,5422} = 19.51$, $p < 0.001$	$F_{3,5681} = 67.03$, $p < 0.001$
ψ_{Mean}	-6.021 $\pm$ 2	-2.473 $\pm$ 7	-3.059 $\pm$ 7	-8.578 $\pm$ 6	$q_{6,891} = 1.624$, $p = 0.659$	$q_{6,1136} = 18.24$, $p < 0.001$	$q_{6,5422} = 31.91$, $p < 0.001$	$F_{3,5681} = 174$, $p < 0.001$
Mid-Upstroke α	114.6 $\pm$ 20	126.3 $\pm$ 18	131.8 $\pm$ 29	146.9 $\pm$ 21	$q_{6,887} = 4.266$, $p = 0.014$	$q_{6,1117} = 14.54$, $p < 0.001$	$q_{6,5074} = 20.95$, $p < 0.001$	$F_{3,5318} = 81.29$, $p < 0.001$
Mid-Downstroke α	43.74 $\pm$ 39	51.6 $\pm$ 34	47.23 $\pm$ 21	37.79 $\pm$ 7	$q_{6,887} = 1.257$, $p = 0.811$	$q_{6,1117} = 13.4$, $p < 0.001$	$q_{6,5074} = 18.01$, $p < 0.001$	$F_{3,5318} = 60.95$, $p < 0.001$
Relative Timing of Supination (t/T)	0.5764 $\pm$ 0.1	0.5344 $\pm$ 0.1	0.5071 $\pm$ 0.1	0.5329 $\pm$ 0.05	$q_{6,888} = 2.319$, $p = 0.356$	$q_{6,1133} = 0.3858$, $p = 0.993$	$q_{6,5293} = 13.3$, $p < 0.001$	$F_{3,5550} = 39.12$, $p < 0.001$
Relative Timing of Downstroke End (t/T)	0.5699 $\pm$ 0.02	0.5503 $\pm$ 0.03	0.5169 $\pm$ 0.03	0.5427 $\pm$ 0.03	$q_{6,891} = 3.629$, $p = 0.051$	$q_{6,1136} = 4.785$, $p = 0.004$	$q_{6,5422} = 31.33$, $p < 0.001$	$F_{3,5681} = 239.4$, $p < 0.001$
Wingbeat Frequency (Hz)	141.5 $\pm$ 3	139.7 $\pm$ 7	137.3 $\pm$ 13	139.1 $\pm$ 7	$q_{6,891} = 0.8425$, $p = 0.933$	$q_{6,1136} = 0.9913$, $p = 0.897$	$q_{6,5422} = 5.623$, $p < 0.001$	$F_{3,5681} = 7.972$, $p < 0.001$

Table B.2: Kinematics of the wings during control flights across different gears compared with *PWP*-removed wingbeats, presented as the mean $\pm$ one standard deviation and the resultant *F*- and *p*-values resulting from a one-way ANOVA test. The *q*-values resulting from Tukey-Kramer comparison tests between different gears and experimental treatments are also presented. Bold values indicate significant results after the application of a false discovery rate algorithm. φ = stroke angle (φ_{Range} therefore represents stroke amplitude), ϑ = deviation angle, α = wing pitch angle, t = time and T = the duration of the whole wingbeat.

Kinematic	Treatment				Comparison with PWP Removed			F-Value
	Gear 1	Gear 2	Gear 3	PWP Removed	Gear 1	Gear 2	Gear 3	
φ_{Max}	23.1 $\pm$ 4	30.92 $\pm$ 13	54.23 $\pm$ 15	31.57 $\pm$ 15	$q_{6,3461} = 2.416$, $p = 0.319$	$q_{6,3706} = 0.954$, $p = 0.907$	$q_{6,7992} = 95.48$, $p < 0.001$	$F_{3,8251} = 1593$ $p < 0.001$
φ_{Min}	-79.99 $\pm$ 1	-80.15 $\pm$ 6	-78.29 $\pm$ 8	-74.98 $\pm$ 7	$q_{6,3461} = 2.738$, $p = 0.213$	$q_{6,3706} = 14.52$, $p < 0.001$	$q_{6,7992} = 26.74$, $p < 0.001$	$F_{3,8251} = 134.4$, $p < 0.001$
φ_{Range}	101.8 $\pm$ 5	108.4 $\pm$ 14	131 $\pm$ 15	104 $\pm$ 16	$q_{6,3461} = 0.7496$, $p = 0.952$	$q_{6,3706} = 5.434$, $p < 0.001$	$q_{6,7992} = 106.8$, $p < 0.001$	$F_{3,8251} = 1948$, $p < 0.001$
ϑ_{Max}	15.48 $\pm$ 2	12.18 $\pm$ 7	6.251 $\pm$ 8	10.4 $\pm$ 7	$q_{6,3461} = 2.719$, $p = 0.218$	$q_{6,3706} = 4.88$, $p = 0.003$	$q_{6,7992} = 32.8$, $p < 0.001$	$F_{3,8251} = 204.2$, $p < 0.001$
ϑ_{Min}	-7.37 $\pm$ 2	-7.741 $\pm$ 7	-16.53 $\pm$ 12	-9.764 $\pm$ 7	$q_{6,3461} = 1.016$, $p = 0.89$	$q_{6,3706} = 4.407$, $p = 0.01$	$q_{6,7992} = 42.45$, $p < 0.001$	$F_{3,8251} = 329.7$, $p < 0.001$
ϑ_{Mean}	0.7197 $\pm$ 0.9	0.3148 $\pm$ 3	-3.694 $\pm$ 7	-0.2303 $\pm$ 3	$q_{6,3461} = 0.7477$, $p = 0.952$	$q_{6,3706} = 2.203$, $p = 0.403$	$q_{6,7992} = 40.29$, $p < 0.001$	$F_{3,8251} = 289.2$, $p < 0.001$
ϑ_{Range}	22.85 $\pm$ 2	19.92 $\pm$ 8	22.78 $\pm$ 8	20.16 $\pm$ 9	$q_{6,3461} = 1.165$, $p = 0.843$	$q_{6,3706} = 0.5467$, $p = 0.98$	$q_{6,7992} = 16.77$, $p < 0.001$	$F_{3,8251} = 49.48$, $p < 0.001$
ϕ_{Mean}	-6.021 $\pm$ 2	-2.473 $\pm$ 7	-3.059 $\pm$ 7	3.526 $\pm$ 11	$q_{6,3461} = 4.515$, $p = 0.008$	$q_{6,3706} = 14.38$, $p < 0.001$	$q_{6,7992} = 45.59$, $p < 0.001$	$F_{3,8251} = 352.6$, $p < 0.001$
Mid-Upstroke α	114.6 $\pm$ 20	126.3 $\pm$ 18	131.8 $\pm$ 29	129.6 $\pm$ 22	$q_{6,3185} = 2.461$, $p = 0.303$	$q_{6,3415} = 2.728$, $p = 0.216$	$q_{6,7372} = 5.017$, $p = 0.002$	$F_{3,7616} = 7.77$, $p < 0.001$
Mid-Downstroke α	43.74 $\pm$ 39	51.6 $\pm$ 34	47.23 $\pm$ 21	35.86 $\pm$ 22	$q_{6,3185} = 1.535$, $p = 0.698$	$q_{6,3415} = 15.27$, $p < 0.001$	$q_{6,7372} = 31.45$, $p < 0.001$	$F_{3,7616} = 178.9$, $p < 0.001$
Relative Timing of Supination (t/T)	0.5764 $\pm$ 0.1	0.5344 $\pm$ 0.1	0.5071 $\pm$ 0.1	0.5882 $\pm$ 0.08	$q_{6,3196} = 0.5888$, $p = 0.976$	$q_{6,3441} = 14.53$, $p < 0.001$	$q_{6,7601} = 61.6$, $p < 0.001$	$F_{3,7858} = 633.4$, $p < 0.001$
Relative Timing of Downstroke End (t/T)	0.5699 $\pm$ 0.02	0.5503 $\pm$ 0.03	0.5169 $\pm$ 0.03	0.5297 $\pm$ 0.03	$q_{6,3461} = 5.195$, $p = 0.001$	$q_{6,3706} = 13.66$, $p < 0.001$	$q_{6,7992} = 24.62$, $p < 0.001$	$F_{3,8251} = 166.5$, $p < 0.001$
Wingbeat Frequency (Hz)	141.5 $\pm$ 3	139.7 $\pm$ 7	137.3 $\pm$ 13	160.2 $\pm$ 40	$q_{6,3461} = 2.874$, $p = 0.176$	$q_{6,3706} = 16.2$, $p < 0.001$	$q_{6,7992} = 52.06$, $p < 0.001$	$F_{3,8251} = 457.9$, $p < 0.001$

Appendix C

Transition ANOVA Results

The following tables present the results of one-way analysis of variance (ANOVA) tests comparing wingbeats transitioning between two gears, with wingbeats that have been beating in the respective gears continuously (i.e. in the same gear on the present and previous wingbeats). The mean $\pm$ one standard deviation of measured wing kinematics of each of these wingbeat types is presented, along with the F -value of the performed ANOVA test and the associated p -value. A false discovery rate algorithm (as detailed in the methods section) has also been applied, and the remaining significant results are highlighted in bold text. The q -value and associated p -value from the post-hoc Tukey-Kramer multiple comparison tests between the transitional wingbeats and their respective continuous wingbeats are also shown. Similarly, the comparison results presented in bold text represent significant differences between the continuous gears and the transitional wingbeats.

Table C.1: Kinematics of wingbeats continuously in gears 1 and 2, compared with wingbeats transitioning from gear 1 to 2.

Kinematic	G1	G2	G2 (prev: G1)	Significance	Comparisons	
					G1 G2 (prev:G1)	G2 G2 (prev:G1)
φ_{Max}	-3.292 $\pm$ 11	5.811 $\pm$ 8	7.61 $\pm$ 13	$F_{2,2300} = 231.4$, $p < 0.001$	$q_{3,1594} = 26.38$, $p < 0.001$	$q_{3,944} = 3.206$, $p = 0.06$
φ_{Min}	-82.87 $\pm$ 7	-80.15 $\pm$ 11	-83.81 $\pm$ 9	$F_{2,2300} = 29.32$, $p < 0.001$	$q_{3,1594} = 2.218$, $p = 0.259$	$q_{3,944} = 8.132$, $p < 0.001$
φ_{Range}	77.78 $\pm$ 13	84.8 $\pm$ 15	91.04 $\pm$ 17	$F_{2,2300} = 115.3$, $p < 0.001$	$q_{3,1594} = 18.42$, $p < 0.001$	$q_{3,944} = 8.128$, $p < 0.001$
ϑ_{Max}	14.95 $\pm$ 7	10.95 $\pm$ 7	11.27 $\pm$ 6	$F_{2,2300} = 87.31$, $p < 0.001$	$q_{3,1594} = 10.49$, $p < 0.001$	$q_{3,944} = 0.875$, $p = 0.81$
ϑ_{Min}	-10.76 $\pm$ 5	-9.738 $\pm$ 5	-11.13 $\pm$ 6	$F_{2,2300} = 10.02$, $p < 0.001$	$q_{3,1594} = 1.333$, $p = 0.613$	$q_{3,944} = 4.778$, $p = 0.002$
ϑ_{Mean}	0.02437 $\pm$ 2	-0.4264 $\pm$ 1	-0.8294 $\pm$ 2	$F_{2,2300} = 29.9$, $p < 0.001$	$q_{3,1594} = 9.395$, $p < 0.001$	$q_{3,944} = 4.162$, $p = 0.009$
ϑ_{Range}	25.71 $\pm$ 12	20.69 $\pm$ 12	22.4 $\pm$ 10	$F_{2,2300} = 45.8$, $p < 0.001$	$q_{3,1594} = 5.721$, $p < 0.001$	$q_{3,944} = 2.779$, $p = 0.121$
ϕ_{Mean}	-7.044 $\pm$ 6	-7.939 $\pm$ 5	-10.04 $\pm$ 6	$F_{2,2300} = 28.75$, $p < 0.001$	$q_{3,1594} = 10.43$, $p < 0.001$	$q_{3,944} = 6.866$, $p < 0.001$
Mid-Upstroke α	128.5 $\pm$ 27	122 $\pm$ 26	135.4 $\pm$ 33	$F_{2,2116} = 22$, $p < 0.001$	$q_{3,1522} = 4.815$, $p = 0.002$	$q_{3,799} = 8.653$, $p < 0.001$
Mid-Downstroke α	25.53 $\pm$ 19	23.9 $\pm$ 15	24.47 $\pm$ 10	$F_{2,2116} = 1.963$, $p = 0.141$	$q_{3,1522} = 4.815$, $p = 0.689$	$q_{3,799} = 0.577$, $p = 0.912$
Relative Timing of Supination (t/T)	0.6543 $\pm$ 0.07	0.6809 $\pm$ 0.1	0.6161 $\pm$ 0.1	$F_{2,2180} = 40.92$, $p < 0.001$	$q_{3,1543} = 7.814$, $p < 0.001$	$q_{3,863} = 12.35$, $p < 0.001$
Relative Timing of Downstroke End (t/T)	0.5782 $\pm$ 0.04	0.5776 $\pm$ 0.04	0.5661 $\pm$ 0.04	$F_{2,2300} = 9.198$, $p < 0.001$	$q_{3,1594} = 5.988$, $p < 0.001$	$q_{3,944} = 5.371$, $p < 0.001$
Wingbeat Frequency (Hz)	166.4 $\pm$ 18	150.4 $\pm$ 15	164 $\pm$ 18	$F_{2,2300} = 197$, $p < 0.001$	$q_{3,1594} = 2.685$, $p = 0.139$	$q_{3,944} = 14.61$, $p < 0.001$

Table C.2: Kinematics of wingbeats continuously in gears 1 and 2, compared with wingbeats transitioning from gear 2 to 1.

Kinematic	G1	G2	G1 (prev: G2)	Significance	Comparisons	
					G1 G1 (prev:G2)	G2 G1 (prev:G2)
φ_{Max}	-3.292 $\pm$ 11	5.811 $\pm$ 8	0.9758 $\pm$ 12	$F_{2,2315} = 179.1$, $p < 0.001$	$q_{3,1609} = 8.415$, $p < 0.001$	$q_{3,959} = 8.921$, $p < 0.001$
φ_{Min}	-82.87 $\pm$ 7	-80.15 $\pm$ 11	-80.15 $\pm$ 11	$F_{2,2315} = 24.89$, $p < 0.001$	$q_{3,1609} = 0.6848$, $p = 0.879$	$q_{3,959} = 5.559$, $p < 0.001$
φ_{Range}	77.78 $\pm$ 13	84.8 $\pm$ 15	80.92 $\pm$ 17	$F_{2,2315} = 55.26$, $p < 0.001$	$q_{3,1609} = 4.466$, $p = 0.005$	$q_{3,959} = 5.161$, $p < 0.001$
ϑ_{Max}	14.95 $\pm$ 7	10.95 $\pm$ 7	17.91 $\pm$ 7	$F_{2,2315} = 114.1$, $p < 0.001$	$q_{3,1609} = 8.509$, $p < 0.001$	$q_{3,959} = 18.73$, $p < 0.001$
ϑ_{Min}	-10.76 $\pm$ 5	-9.738 $\pm$ 5	-12.58 $\pm$ 5	$F_{2,2315} = 26.48$, $p < 0.001$	$q_{3,1609} = 6.934$, $p < 0.001$	$q_{3,959} = 10.14$, $p < 0.001$
ϑ_{Mean}	0.02437 $\pm$ 2	-0.4264 $\pm$ 1	0.5615 $\pm$ 2	$F_{2,2315} = 30.29$, $p < 0.001$	$q_{3,1609} = 6.053$, $p < 0.001$	$q_{3,959} = 10.42$, $p < 0.001$
ϑ_{Range}	25.71 $\pm$ 12	20.69 $\pm$ 12	30.49 $\pm$ 12	$F_{2,2315} = 77.22$, $p < 0.001$	$q_{3,1609} = 8.381$, $p < 0.001$	$q_{3,959} = 16.09$, $p < 0.001$
ψ_{Mean}	-7.044 $\pm$ 6	-7.939 $\pm$ 5	-8.388 $\pm$ 5	$F_{2,2315} = 9.445$, $p < 0.001$	$q_{3,1609} = 4.848$, $p = 0.001$	$q_{3,959} = 1.515$, $p = 0.532$
Mid-Upstroke α	128.5 $\pm$ 27	122 $\pm$ 26	128.6 $\pm$ 26	$F_{2,2131} = 13.16$, $p < 0.001$	$q_{3,1537} = 0.07113$, $p = 0.999$	$q_{3,814} = 4.471$, $p = 0.004$
Mid-Downstroke α	25.53 $\pm$ 19	23.9 $\pm$ 15	25.03 $\pm$ 19	$F_{2,2131} = 1.72$, $p = 0.179$	$q_{3,1537} = 0.5455$, $p = 0.921$	$q_{3,814} = 1.128$, $p = 0.705$
Relative Timing of Supination (t/T)	0.6543 $\pm$ 0.07	0.6809 $\pm$ 0.1	0.6168 $\pm$ 0.1	$F_{2,2188} = 40.95$, $p < 0.001$	$q_{3,1551} = 7.796$, $p < 0.001$	$q_{3,871} = 12.38$, $p < 0.001$
Relative Timing of Downstroke End (t/T)	0.5782 $\pm$ 0.04	0.5776 $\pm$ 0.04	0.5593 $\pm$ 0.04	$F_{2,2315} = 23.71$, $p < 0.001$	$q_{3,1609} = 9.561$, $p < 0.001$	$q_{3,959} = 8.692$, $p < 0.001$
Wingbeat Frequency (Hz)	166.4 $\pm$ 18	150.4 $\pm$ 15	171.1 $\pm$ 17	textitF _{2,2315} = 233.6, $p < 0.001$	$q_{3,1609} = 5.522$, $p < 0.001$	$q_{3,959} = 22.79$, $p < 0.001$

Table C.3: Kinematics of wingbeats continuously in gears 1 and 3, compared with wingbeats transitioning from gear 1 to 3.

Kinematic	G1	G3	G3 (prev: G1)	Significance	Comparisons	
					G1 G3 (prev:G1)	G3 G3 (prev:G1)
φ_{Max}	-3.292 $\pm$ 11	19.15 $\pm$ 13	12.07 $\pm$ 11	$F_{2,4631} = 1474$, $p < 0.001$	$q_{3,1506} = 19.66$, $p < 0.001$	$q_{3,3275} = 9.336$, $p < 0.001$
φ_{Min}	-82.87 $\pm$ 7	-83.2 $\pm$ 10	-83.84 $\pm$ 6	$F_{2,4631} = 1.003$, $p = 0.367$	$q_{3,1506} = 1.68$, $p = 0.46$	$q_{3,3275} = 1.155$, $p = 0.693$
φ_{Range}	77.78 $\pm$ 13	101 $\pm$ 18	94.43 $\pm$ 15	$F_{2,4631} = 880.7$, $p < 0.001$	$q_{3,1506} = 15.93$, $p < 0.001$	$q_{3,3275} = 6.459$, $p < 0.001$
ϑ_{Max}	14.95 $\pm$ 7	8.227 $\pm$ 7	10.71 $\pm$ 8	$F_{2,4631} = 419.5$, $p < 0.001$	$q_{3,1506} = 9.659$, $p < 0.001$	$q_{3,3275} = 5.827$, $p < 0.001$
ϑ_{Min}	-10.76 $\pm$ 5	-14.16 $\pm$ 8	-11.66 $\pm$ 7	$F_{2,4631} = 105.7$, $p < 0.001$	$q_{3,1506} = 1.998$, $p = 0.334$	$q_{3,3275} = 5.751$, $p < 0.001$
ϑ_{Mean}	0.02437 $\pm$ 2	-2.726 $\pm$ 4	-1.063 $\pm$ 2	$F_{2,4631} = 259.5$, $p < 0.001$	$q_{3,1506} = 4.735$, $p = 0.002$	$q_{3,3275} = -7.447$, $p < 0.001$
ϑ_{Range}	25.71 $\pm$ 12	22.39 $\pm$ 11	22.37 $\pm$ 14	$F_{2,4631} = 43.29$, $p < 0.001$	$q_{3,1506} = 4.915$, $p = 0.001$	$q_{3,3275} = 0.02654$, $p = 1$
ϕ_{Mean}	-7.044 $\pm$ 6	-5.468 $\pm$ 6	-8.469 $\pm$ 6	$F_{2,4631} = 48.9$, $p < 0.001$	$q_{3,1506} = 4.02$, $p = 0.012$	$q_{3,3275} = 8.71$, $p < 0.001$
Mid-Upstroke α	128.5 $\pm$ 27	145.4 $\pm$ 22	139.4 $\pm$ 22	$F_{2,4001} = 220.9$, $p < 0.001$	$q_{3,1460} = 7.339$, $p < 0.001$	$q_{3,2684} = 4.1$, $p = 0.01$
Mid-Downstroke α	25.53 $\pm$ 19	26.04 $\pm$ 13	28.12 $\pm$ 13	$F_{2,4001} = 2.003$, $p = 0.135$	$q_{3,1460} = 2.278$, $p = 0.13$	$q_{3,2684} = 2.241$, $p = 0.252$
Relative Timing of Supination (t/T)	0.6543 $\pm$ 0.07	0.6506 $\pm$ 0.07	0.6335 $\pm$ 0.07	$F_{2,4038} = 5.411$, $p = 0.005$	$q_{3,1460} = 4.548$, $p = 0.004$	$q_{3,2721} = 3.843$, $p = 0.018$
Relative Timing of Downstroke End (t/T)	0.5782 $\pm$ 0.04	0.5613 $\pm$ 0.03	0.5605 $\pm$ 0.04	$F_{2,4631} = 121.3$, $p < 0.001$	$q_{3,1506} = 8.564$, $p < 0.001$	$q_{3,3275} = 0.4103$, $p = 0.955$
Wingbeat Frequency (Hz)	166.4 $\pm$ 18	156.8 $\pm$ 14	156.4 $\pm$ 23	$F_{2,4631} = 172.4$, $p < 0.001$	$q_{3,1506} = 10.15$, $p < 0.001$	$q_{3,3275} = 0.4103$, $p = 0.951$

Table C.4: Kinematics of wingbeats continuously in gears 1 and 3, compared with wingbeats transitioning from gear 3 to 1.

Kinematic	G1	G3	G1 (prev: G3)	Significance	Comparisons	
					G1 G1 (prev:G3)	G3 G1 (prev:G3)
φ_{Max}	-3.292 ± 11	19.15 ± 13	0.07252 ± 11	$F_{2,4620} = 1534$, $p < 0.001$	$q_{3,1495} = 4.155$, $p = 0.009$	$q_{3,3264} = 24.2$, $p < 0.001$
φ_{Min}	-82.87 ± 7	-83.2 ± 10	-82.18 ± 7	$F_{2,4620} = 1.177$, $p = 0.308$	$q_{3,1495} = 1.154$, $p = 0.693$	$q_{3,3264} = 1.737$, $p = 0.437$
φ_{Range}	77.78 ± 13	101 ± 18	78.37 ± 14	$F_{2,4620} = 936.9$, $p < 0.001$	$q_{3,1495} = 0.5478$, $p = 0.921$	$q_{3,3264} = 21.46$, $p < 0.001$
ϑ_{Max}	14.95 ± 7	8.227 ± 7	17.17 ± 7	$F_{2,4620} = 484.8$, $p < 0.001$	$q_{3,1495} = 4.9$, $p = 0.002$	$q_{3,3264} = 20.28$, $p < 0.001$
ϑ_{Min}	-10.76 ± 5	-14.16 ± 8	-13.12 ± 5	$F_{2,4620} = 104.2$, $p < 0.001$	$q_{3,1495} = 5.112$, $p < 0.001$	$q_{3,3264} = 2.323$, $p = 0.2278$
ϑ_{Mean}	0.02437 ± 2	-2.726 ± 4	0.0853 ± 2	$F_{2,4620} = 275$, $p < 0.001$	$q_{3,1495} = 0.2564$, $p = 0.982$	$q_{3,3264} = 12.14$, $p < 0.001$
ϑ_{Range}	25.71 ± 12	22.39 ± 11	30.28 ± 11	$F_{2,4620} = 69.3$, $p < 0.001$	$q_{3,1495} = 6.541$, $p < 0.001$	$q_{3,3264} = 11.6$, $p < 0.001$
ψ_{Mean}	-7.044 ± 6	-5.468 ± 6	-5.992 ± 7	$F_{2,4620} = 34.91$, $p < 0.001$	$q_{3,1495} = 2.848$, $p = 0.109$	$q_{3,3264} = 1.458$, $p = 0.557$
Mid-Upstroke α	128.5 ± 27	145.4 ± 22	122.2 ± 27	$F_{2,3989} = 251.3$, $p < 0.001$	$q_{3,1448} = 4.009$, $p = 0.013$	$q_{3,2672} = 15.17$, $p < 0.001$
Mid-Downstroke α	25.53 ± 19	26.04 ± 13	30.36 ± 26	$F_{2,3989} = 5.577$, $p = 0.004$	$q_{3,1448} = 4.717$, $p = 0.002$	$q_{3,2672} = 4.309$, $p = 0.007$
Relative Timing of Supination (t/T)	0.6543 ± 0.07	0.6506 ± 0.07	0.6581 ± 0.09	$F_{2,4026} = 1.515$, $p = 0.22$	$q_{3,1448} = 0.7883$, $p = 0.843$	$q_{3,2709} = 1.58$, $p = 0.503$
Relative Timing of Downstroke End (t/T)	0.5782 ± 0.04	0.5613 ± 0.03	0.5603 ± 0.04	$F_{2,4620} = 120.6$, $p < 0.001$	$q_{3,1495} = 8.325$, $p < 0.001$	$q_{3,3264} = 0.4755$, $p = 0.94$
Wingbeat Frequency (Hz)	166.4 ± 18	156.8 ± 14	169.5 ± 22	$F_{2,4620} = 120.6$, $p < 0.001$	$q_{3,1495} = 3.057$, $p = 0.078$	$q_{3,3264} = 12.83$, $p < 0.001$

Table C.5: Kinematics of wingbeats in gears 2 and 3, compared with wingbeats transitioning from gear 2 to 3.

Kinematic	G2	G3	G3 (prev: G2)	Significance	Comparisons	
					G2 G3 (prev:G2)	G3 G3 (prev:G2)
φ_{Max}	5.811 $\pm$ 8	19.15 $\pm$ 13	16.62 $\pm$ 10	$F_{2,4199} = 335.4$, $p < 0.001$	$q_{3,1074} = 19.16$, $p < 0.001$	$q_{3,3493} = 5.225$, $p < 0.001$
φ_{Min}	-80.15 $\pm$ 11	-83.2 $\pm$ 10	-83.41 $\pm$ 9	$F_{2,4199} = 26.09$, $p < 0.001$	$q_{3,1074} = 6.934$, $p < 0.001$	$q_{3,3493} = 0.5338$, $p = 0.924$
φ_{Range}	84.8 $\pm$ 15	101 $\pm$ 18	94.71 $\pm$ 16	$F_{2,4199} = 240.6$, $p < 0.001$	$q_{3,1074} = 17.2$, $p < 0.001$	$q_{3,3493} = 3.283$, $p = 0.053$
ϑ_{Max}	10.95 $\pm$ 7	8.227 $\pm$ 7	10.4 $\pm$ 7	$F_{2,4199} = 52.46$, $p < 0.001$	$q_{3,1074} = 1.703$, $p = 0.451$	$q_{3,3493} = 7.888$, $p < 0.001$
ϑ_{Min}	-9.738 $\pm$ 5	-14.16 $\pm$ 8	-10.56 $\pm$ 5	$F_{2,4199} = 127.3$, $p < 0.001$	$q_{3,1074} = 2.453$, $p = 0.192$	$q_{3,3493} = 12.45$, $p < 0.001$
ϑ_{Mean}	-0.4264 $\pm$ 1	-2.726 $\pm$ 4	-1.028 $\pm$ 1	$F_{2,4199} = 123.4$, $p < 0.001$	$q_{3,1074} = 3.4623$, $p = 0.038$	$q_{3,3493} = 11.37$, $p < 0.001$
ϑ_{Range}	20.69 $\pm$ 12	22.39 $\pm$ 11	20.96 $\pm$ 11	$F_{2,4199} = 8.888$, $p < 0.001$	$q_{3,1074} = 0.5651$, $p = 0.916$	$q_{3,3493} = 3.361$, $p = 0.046$
ϕ_{Mean}	-7.939 $\pm$ 5	-5.468 $\pm$ 6	-7.547 $\pm$ 4	$F_{2,4199} = 78.89$, $p < 0.001$	$q_{3,1074} = 1.629$, $p = 0.482$	$q_{3,3493} = 10.06$, $p < 0.001$
Mid-Upstroke α	122 $\pm$ 21	145.4 $\pm$ 22	145 $\pm$ 21	$F_{2,3464} = 261.8$, $p < 0.001$	$q_{3,923} = 20.85$, $p < 0.001$	$q_{3,2870} = 0.3783$, $p = 0.961$
Mid-Downstroke α	23.9 $\pm$ 15	26.04 $\pm$ 13	27.5 $\pm$ 12	$F_{2,3464} = 9.559$, $p < 0.001$	$q_{3,923} = 5.676$, $p < 0.001$	$q_{3,2870} = 2.695$, $p = 0.137$
Relative Timing of Supination (t/T)	0.6809 $\pm$ 0.1	0.6506 $\pm$ 0.07	0.6446 $\pm$ 0.06	$F_{2,3547} = 37.49$, $p < 0.001$	$q_{3,969} = 9.15$, $p < 0.001$	$q_{3,2910} = 1.766$, $p = 0.424$
Relative Timing of Downstroke End (t/T)	0.5776 $\pm$ 0.04	0.5613 $\pm$ 0.03	0.5758 $\pm$ 0.03	$F_{2,4199} = 95.9$, $p < 0.001$	$q_{3,1074} = 1.264$, $p = 0.644$	$q_{3,3493} = 11.53$, $p < 0.001$
Wingbeat Frequency (Hz)	150.4 $\pm$ 15	156.8 $\pm$ 14	156.8 $\pm$ 19	$F_{2,4199} = 54.41$, $p < 0.001$	$q_{3,1074} = 9.42$, $p < 0.001$	$q_{3,3493} = 0.01665$, $p = 1$

Table C.6: Kinematics of wingbeats continuously in gears 2 and 3, compared with wingbeats transitioning from gear 3 to 2.

Kinematic	G2	G3	G2 (prev: G3)	Significance	Comparisons	
					G2 G2 (prev:G3)	G3 G2 (prev:G3)
φ_{Max}	5.811 $\pm$ 8	19.15 $\pm$ 13	5.837 $\pm$ 9	$F_{2,4207} = 468.6$, $p < 0.001$	$q_{3,1082} = 0.04513$, $p = 0.999$	$q_{3,3501} = 27.84$, $p < 0.001$
φ_{Min}	-80.15 $\pm$ 11	-83.2 $\pm$ 10	-82.24 $\pm$ 9	$F_{2,4207} = 25.3$, $p < 0.001$	$q_{3,1082} = 4.475$, $p = 0.004$	$q_{3,3501} = 2.378$, $p = 0.212$
φ_{Range}	84.8 $\pm$ 15	101 $\pm$ 18	86.4 $\pm$ 16	$F_{2,4207} = 312.8$, $p < 0.001$	$q_{3,1082} = 1.994$, $p = 0.336$	$q_{3,3501} = 21.21$, $p < 0.001$
ϑ_{Max}	10.95 $\pm$ 7	8.227 $\pm$ 7	13.25 $\pm$ 6	$F_{2,4207} = 115.5$, $p < 0.001$	$q_{3,1082} = 7.301$, $p < 0.001$	$q_{3,3501} = 18.6$, $p < 0.001$
ϑ_{Min}	-9.738 $\pm$ 5	-14.16 $\pm$ 8	-11.37 $\pm$ 4	$F_{2,4207} = 117.1$, $p < 0.001$	$q_{3,1082} = 4.921$, $p = 0.001$	$q_{3,3501} = 9.804$, $p < 0.001$
ϑ_{Mean}	-0.4264 $\pm$ 1	-2.726 $\pm$ 4	-0.6458 $\pm$ 1	$F_{2,4207} = 137.4$, $p < 0.001$	$q_{3,1082} = 1.274$, $p = 0.64$	$q_{3,3501} = 14.1$, $p < 0.001$
ϑ_{Range}	20.69 $\pm$ 12	22.39 $\pm$ 11	24.62 $\pm$ 9	$F_{2,4207} = 17$, $p < 0.001$	$q_{3,1082} = 8.145$, $p < 0.001$	$q_{3,3501} = 5.401$, $p < 0.001$
ϕ_{Mean}	-7.939 $\pm$ 5	-5.468 $\pm$ 6	-6.104 $\pm$ 5	$F_{2,4207} = 62.06$, $p < 0.001$	$q_{3,1082} = 7.595$, $p < 0.001$	$q_{3,3501} = 3.077$, $p = 0.075$
Mid-Upstroke α	122 $\pm$ 21	145.4 $\pm$ 22	139.2 $\pm$ 21	$F_{2,3471} = 257.1$, $p < 0.001$	$q_{3,930} = 15.61$, $p < 0.001$	$q_{3,2877} = 6.659$, $p < 0.001$
Mid-Downstroke α	23.9 $\pm$ 15	26.04 $\pm$ 13	27.71 $\pm$ 12	$F_{2,3471} = 10.25$, $p < 0.001$	$q_{3,930} = 6.015$, $p < 0.001$	$q_{3,2877} = 3.086$, $p = 0.074$
Relative Timing of Supination (t/T)	0.6809 $\pm$ 0.1	0.6506 $\pm$ 0.07	0.654 $\pm$ 0.07	$F_{2,3555} = 34.42$, $p < 0.001$	$q_{3,977} = 6.826$, $p < 0.001$	$q_{3,2918} = 0.9934$, $p = 0.762$
Relative Timing of Downstroke End (t/T)	0.5776 $\pm$ 0.04	0.5613 $\pm$ 0.03	0.5754 $\pm$ 0.04	$F_{2,4207} = 93.45$, $p < 0.001$	$q_{3,1082} = 1.485$, $p = 0.545$	$q_{3,3501} = 11.28$, $p < 0.001$
Wingbeat Frequency (Hz)	150.4 $\pm$ 15	156.8 $\pm$ 14	165.6 $\pm$ 19	$F_{2,4207} = 128.2$, $p < 0.001$	$q_{3,1082} = 22.41$, $p < 0.001$	$q_{3,3501} = 15.07$, $p < 0.001$

Chapter 6

The Role of the Pterale C

Proprioception is vitally important for the maintenance of stability in animals, particularly during flight. Proprioceptors that determine the position of the wing relative to the body allow insects to identify disturbances to the expected pattern of wing movement, and compensate appropriately to maintain equilibrium. Many insects have sensory organs within the wing hinge which inform the central nervous system (CNS) of the approach of the end of each half stroke, but no such organ has been conclusively identified in Diptera. A poorly understood structure, the Pterale C, has been suggested to fill this role of wingbeat-synchronous proprioceptor, but its function remains a matter of dispute. Using high speed macrophotography, we document the movement of the Pterale C during the wingbeat. By documenting contact between this component and a part of the wing's radial vein as the wing nears the transition from being posterior to anterior of the wing hinge, we find a strong correlation between the timing of this event, and the time of wing reversal at the end of the downstroke. We thereby suggest that the Pterale C may be responsible for informing the CNS of the position of the wing as it approaches the end of the downstroke. We also find evidence against the 'shock absorber' hypothesis, in which the contact between the Pterale C and radial vein would restrict wing movement and absorb kinetic energy. Our study here has made use of modern technologies to contribute towards a long-standing argument, and has highlighted the clear importance of wing-synchronous proprioception in allowing dipteran insects to control

their wings with such precision.

6.1 Introduction

To help stabilise their movements, animals make use of sensory organs to inform their central nervous systems (CNS) of the position of their limbs. These proprioceptors detect the position of the limbs relative to the body, and allow the animal in question to compensate for any unexpected perturbations of limb motion. Proprioceptive organs thereby allow mobile animals to maintain stability in the face of environmental influences which would otherwise disturb their equilibrium.

Due to the variety of different lifestyles exhibited by animals, these proprioceptive organs are highly specialised. As a result, these organs are able to provide highly useful information to the animal's CNS. Examples of these organs include the femoral chordotonal organ in insects (e.g. Bässler (1965); Usherwood et al. (1968); Clarac et al. (1978); Field and Burrows (1982)), and the vestibular system in vertebrates (Keshner and Cohen, 1989a). These components are often responsible for eliciting reactions in response to movements of certain parts of the body. The femoral chordotonal organs in locusts, for example, elicit muscle activity that causes flexion of the tibia in response to its extension, thereby aiding the stability of the femoro-tibial joint. Similarly, the vertebrate vestibular system can excite muscles of the neck and limbs in response to movements of the head in efforts to stabilise it in a vertical position (e.g. Schor (1974); Peterson et al. (1985); Keshner and Cohen (1989b)).

Proprioceptive organs are particularly important for animals that undergo fast rates of movement, as they must make rapid locomotor adjustments to avoid exacerbating unwanted changes to their limb movements. This is especially important for animals of small sizes which are more easily affected by environmental disturbances due to their small weight and inertia. Insects are subject to both of these conditions, and are therefore particularly reliant on highly informative sensory organs.

One famous example of such organs are the halteres of dipteran insects. The bases of these highly modified hindwings are covered with proprioceptive organs

known as campaniform sensilla, which are sensitive to cuticular stress (Pringle, 1948; Gettrup, 1965; Nalbach, 1993; Fayyazuddin and Dickinson, 1996; Fox et al., 2010; Bartussek et al., 2013). During flight, the club-like head of the haltere swings in antiphase to the wings, and has enough inertia for the organ to twist and bend during flight and manoeuvres. The campaniform fields at the base of the organ then detect Coriolis forces generated by its beating in a non-inertial axis system. Through the use of proprioception, these organs are thereby able to inform the CNS of whole body movements, and allow it to respond appropriately. Analogous forewing halteres may be present in the Strepsiptera, but these have received comparably little study (Pix et al., 1993).

These same campaniform sensillae are found on the forewings of the Diptera and other orders of insects (such as the Hymenoptera, Orthoptera and Lepidoptera), both around the base and along the veins of the wing. The campaniform fields are therefore able to detect torsion and camber of the wing, as well as inform the CNS of the position of the wing base relative to the thorax (Gettrup, 1965; Schmidt and Smith, 1985; Gnatzy et al., 1987; Elson, 1987; Dickinson, 1990, 1992; Gray and Robertson, 1994; Dickerson et al., 2014).

Combined with the movement of the halteres, these campaniform fields are thought to provide information regarding the timing of different stages of the wing-beat, such as the beginning or end of the upstroke and downstroke. However, several insects have additional organs within the wing hinge which are thought to be specifically adapted for this role. For example, the locust tegula becomes stimulated as the wing approaches the end of the downstroke (Kien and Altman, 1979; Pearson et al., 1989; Büschges and Pearson, 1991; Wolf, 1993; Gray and Robertson, 1994; Stevenson, 1997; Fischer and Ebert, 1999), while the hawkmoth, *Manduca sexta*, has a wing hinge stretch receptor which elicits action potentials as the hinge is strained during the latter stages of the upstroke (Yack, 1992; Yack and Fullard, 1993; Frye, 2001). However, no such organ has been conclusively identified as performing the same role in the Diptera.



Figure 6.1: A scanning electron microscope image showing the detail of the right wing hinge of *Calliphora vicina*. Anterior is to the right. Abbreviations: *PtC* - Pterale C; *PWP* - Pleural Wing Process; *RS* - Radial Stop; *RV* - Radial Vein.

One candidate for this role of wing hinge proprioceptor is the poorly understood exoskeletal component at the base of the wing, the Pterale C (Ritter, 1911). The head of the *PtC* fits neatly into a groove on the anterior edge of the radial stop (*RS*; Chowdhury and Parr (1981); Miyan and Ewing (1984)), and one of the few studies to focus explicitly on this organ recorded nervous activity at the base of the *PtC* in response to the movement of the whole structure by puffs of air (Miyan and Ewing, 1984). Such evidence has been used to support a hypothesis that the *PtC* is a wingbeat-synchronous proprioceptor. This conclusion has been used to explain bilateral asymmetries in wing kinematics such as wing supination observed in Nalbach's study of the gear change mechanism (Nalbach, 1989), and has been included in a detailed analysis of the wing hinge by Miyan and Ewing (Miyan and Ewing, 1985a).

Other authors, however, have focused on the extensive contact of the *PtC* with the radial stop (*RS*) on the wing's radial vein. Rather than a sensory structure, these authors assert that the *PtC* is a shock-absorber, and that its contact with

the *RS* allows the *PtC* to control the anterior movement of the wing (Chowdhury and Parr, 1981; Wisser, 1988; Nalbach, 1989). In this case, the *PtC* would not be sensory, and would instead act to absorb kinetic energy to control wing kinematics in a similar manner to the *RS*-Pleural Wing Process interaction that forms the gear change mechanism (Chapter 3 (Pfau, 1973, 1987; Ennos, 1987; Wisser, 1988; Nalbach, 1989; Deora et al., 2015)). The prominence of the *PtC* and its contact with the *RS* suggests that elucidating its role is important for fully understanding how dipteran insects control their wings.

Here we use high-speed macrophotography to visualise the interaction of the *RS* and *PtC* of the blowfly (*Calliphora vicina*) during tethered flight in real time, and use three-dimensional reconstructions of the wing kinematics to determine any effects of this contact on wing movement. In doing so, we have been able to observe the movement of the *PtC* throughout the wingbeat in detail which has previously not been possible, and identify how *RS-PtC* contact influences wing kinematics. Our results reveal that the *RS* and *PtC* make extensive contact during the later portions of the downstroke, and that the *PtC* can move independently of the wing and wing hinge. We also show that the timing of *RS-PtC* contact correlates strongly with the timing of the end of the downstroke, and find no evidence of this contact inhibiting wing movement. From this, we suggest that the *PtC* is indeed a proprioceptor, responsible for informing the CNS of the approach of the end of the downstroke.

Like the locust tegula and hawkmoth wing hinge stretch receptor, our results support the hypothesis that dipteran insects possess a highly adapted wingbeat-synchronous proprioceptor within the wing hinge. We assert that the *PtC* may be responsible for providing information regarding the position and movement of the wing towards the end of the downstroke, thereby allowing these animals to react accordingly during flight and maintain fine control over wing and body kinematics.

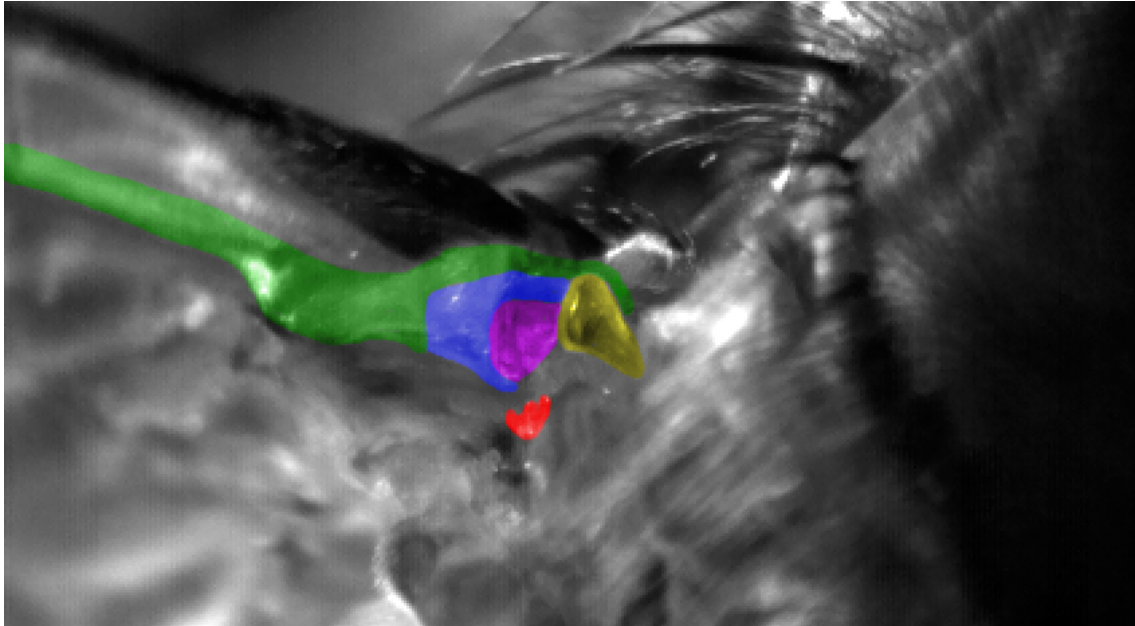


Figure 6.2: A magnified still image taken from a high speed video of the wing hinge, illustrating various components. Green - Radial vein of the wing; Yellow - Pterale C; Red - Pleural Wing Process; Blue - Radial Stop; Purple - Anterior groove of the radial stop (into which the *PtC* fits).

6.2 Materials and Methods

Unless otherwise specified, the insect preparation, high speed camera set up, wing-beat gear classification, and methods of three-dimensional wing kinematic measurement are the same as those used to study the gear change mechanism of *C. vicina* in Chapter 3. We also collected μ CT images using the same methodology as that found in Chapter A.1.

6.2.1 Pterale C Contact Timing

Using the high-speed camera videos (filmed at 6,400 frames per second, giving a resolution of 0.15ms) of the wing hinge, we determined the time during the wingbeat at which the *PtC* made contact with the groove found on the anterior side of the *RS* (figure 6.2). Due to the angle of the camera view it was sometimes difficult to determine the exact frame in which contact was made. In this event, the wingbeat was not included in the final analysis.

6.2.2 Statistical Analyses

We examined any differences across gears in the timing of *RS-PtC* contact and the position of the wing at the moment using one-way ANOVA tests. Further to this, we used Tukey-Kramer comparison tests to identify any significant variations between individual gears.

To study the effects of this contact on other wing kinematics, we built three separate linear regression models in MATLAB to identify any relationships between the timing *RS-PtC* contact with three features of the wingbeat: the timing of the downstroke's end, the timing of wing supination, and the maximum stroke angle reached at the end of the downstroke. We constructed these models using the timing of *RS-PtC* contact as a continuous predictor in each of these models, the gear engaged during the wingbeat as a categorical variable, and an interaction term between these two variables. We then used one-way ANOVA tests to identify the significance of each term within these linear models.

6.3 Results

6.3.1 Timing of Radial Stop-Pterale C Contact

We collected data on the timing of *RS-PtC* contact over 1,052 wingbeats, 175 of which were engaged in gear 1, 100 in gear 2, and 777 of which were engaged in gear 3. The *PtC* made contact with a groove in the anterior side of the *RS* towards the end of the downstroke on every wingbeat (figure 6.3), though the precise timing of this contact varied across wingbeats ($t_{PtC}/T = 0.4307 \pm 0.07$, and $t_{Down}/T = 0.5792 \pm 0.03$ mean $\pm$ one standard deviation; t_{PtC} is the time of *RS-PtC* contact, t_{Down} is the time of the end of the downstroke, and T is the total duration of the wingbeat, where t is equal to zero at the start of the downstroke). This was in part due to variations in the path of the *RS*, but also due to changes in the position of the *PtC* itself. The *PtC* was often seen to move independently of other components of the wing hinge (supplementary video 6.1), indicating some muscular control over

the position and orientation of the organ. This movement is likely to influence the timing of *RS-PtC* contact independently of changes in wing movement.

In the same way that the timing of *RS-PtC* contact varies, as does the position of the wing at the moment of this contact (figure 6.3). Excluding outliers (defined as those points outside three scaled median absolute deviations from the median) the range of stroke angles at the moment of *RS-PtC* contact is 50.19° , while the interquartile range is 11.21° . An ANOVA test reveals that the mean position of the wing at this point of contact also appears to vary between gears ($F_{2,1044} = 65.95$, $p < 0.001$), with there being significant differences between the stroke angle of the wing during gear 3 ($t/T = -8.154 \pm 11^\circ$, mean $\pm$ one standard deviation) and during both gears 1 and 2 ($t/T = -16.83 \pm 8^\circ$ and $t/T = -14.4 \pm 7^\circ$ for gears 1 and 2, respectively; $q_{3,952} = 14.95$, $p < 0.001$; $q_{3,877} = 8.474$, $p < 0.001$, comparing gear 3 to gears 1 and 2, respectively). However, the mean stroke angle of the wing at the point of *RS-PtC* contact does not differ significantly between gears 1 and 2 ($q_{3,275} = 2.799$, $p = 0.117$). This suggests that the contact of the *RS* and *PWP* during gears 1 and 2 causes the former to contact the *PtC* at a more posterior stroke angle than in gear 3 where there is no contact, which may affect the role that the *PtC* plays during these differently geared wingbeats.

Regardless of the differences between different gears, the stroke angle at the moment of *RS-PtC* contact is less than 0° for the majority of wingbeats (947 out of 1054, or 89.85% of wingbeats), meaning that the wingtip was posterior of the wing hinge when the *RS* and *PtC* made contact on the vast majority of wingbeats. Furthermore, 38.99% wingbeats showed *RS-PtC* contact at a stroke angle between 0° and -10° , and 72.87% wingbeats exhibited contact between 0° and -20° , which suggests that this contact tends to occur very shortly before the wingtip transitions from being posterior to being anterior of the wing hinge.

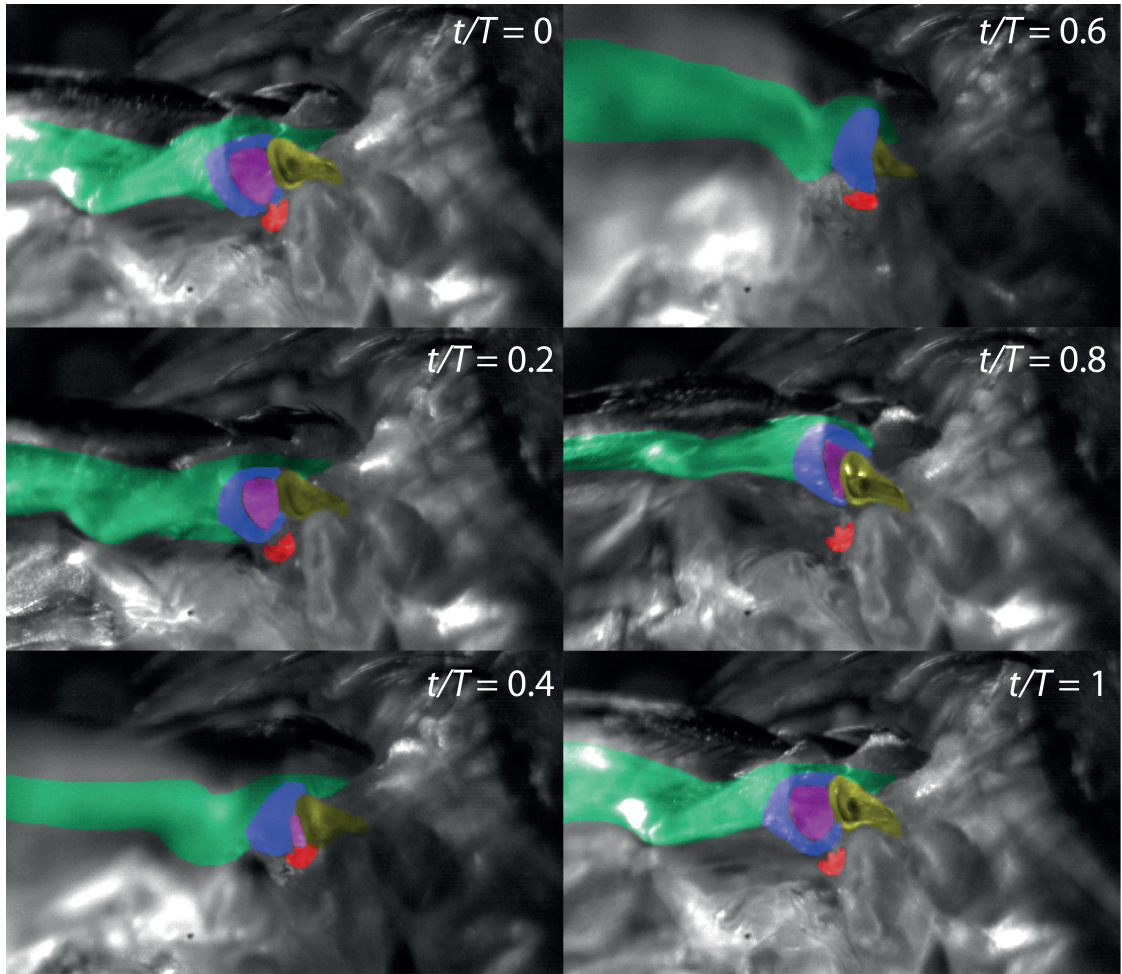


Figure 6.3: A sequence of frames taken from a high-speed video of the wing hinge of *C. vicina*, illustrating the contact between the *RS* and *PtC*. Note the contact with the *PtC* and the groove of the *RS* at $t/T = 0.4$, and the obfuscation of the *PtC* by the *RS* at $t/T = 0.6$ as the *PtC* slots into the anterior groove of the *RS*. t is time and T is the duration of the whole wingbeat, where t is equal to zero at the start of the downstroke. Blue - Radial stop; Green - Radial vein; Purple - Anterior groove of the radial stop; Red - Pleural wing process; Yellow - Pterale C.

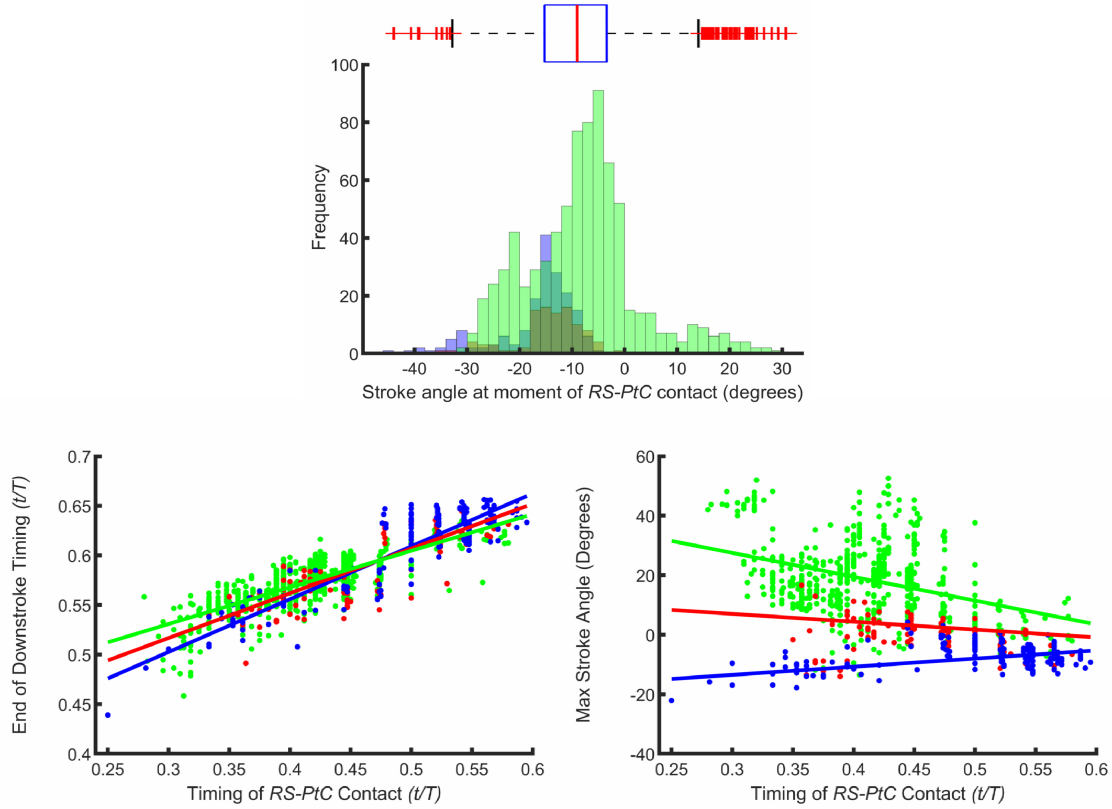


Figure 6.4: Top: A histogram illustrating the stroke angle of the wing at the moment of *RS-PtC* contact. The boxplot above the histogram shows the range of the data excluding outliers (black bars), the interquartile range (blue box), the median (red) line, and outliers (red crosses) of the whole dataset when not separated by gear. Green bars - gear 3 wingbeats; red bars - gear 2 wingbeats; blue bars - gear 1 wingbeats. Bottom: Scatter plots of *RS-PtC* contact timing versus stroke reversal timing (left) and maximum stroke angle (right) across different gears. Points are coloured according to gear: Green - gear 3, $n = 777$; red - gear 2, $n = 100$; blue - gear 1, $n = 175$. Best-fit regression lines are plotted separately for each gear in their respective colours.

6.3.2 Correlates of Radial Stop-Pterale C Contact

The linear model predicting the timing of the end of the downstroke found the timing of *RS-PtC* contact to be a significant positive predictor independent of the gear engaged during the wingbeat (figure 6.4 & table 6.1; appendix D), with the model explaining 74.2% of the total variation in the end of downstroke timing ($R^2 = 0.742$, $F_{3,1043} = 1000$, $p < 0.001$). The timing of *RS-PtC* contact is also a significant predictor in the linear model predicting the timing of wing supination, showing a positive correlation between the two, though this model explains much less of the variation compared to the model predicting the end of the downstroke, and the effect of *RS-PtC* contact is influenced by the gear engaged ($R^2 = 0.192$, $F_{3,1034} = 83.8$, $p < 0.001$). Finally, we found a significant, but weaker, negative correlation

between the timing of *PtC* contact and the maximum anterior stroke angle reached ($R^2 = 0.592$, $F_{3,1043} = 504$, $p < 0.001$). In this final model, the categorical gear engagement variable was also a significant predictor (as would be expected from the results presented in Chapter 3) and there was a significant interaction between these two variables. These results suggest that *RS-PtC* contact occurs earlier in the wingbeat when the duration of the downstroke is shorter and wing supination occurs earlier, but this contact occurs later when the maximum stroke angle is greater.

A mechanical role of the *PtC* would not explain the positive correlation seen with the timing of contact and the time of wing reversal at the end of the downstroke. A shock absorbing role would also presumably lead to a positive correlation between the maximum stroke angle and the timing of *PtC* contact, whereas we see a negative correlation. We therefore conclude that the *PtC* has no mechanical role in determining the movement of the wing, and may instead have some proprioceptive role.

Although we have identified a relationship between the timing of *RS-PtC* contact with the timing of the end of the downstroke and the time of wing supination, there is a large amount of variation in the latency between *RS-PtC* contact and both of these wing kinematic parameters (figure 6.5). Between the timing of *RS-PtC* contact and the end of the downstroke, there is a total range of $t/T = 0.2457$ and an interquartile range of $t/T = 0.0587$, not including outliers. While between the timing of this contact and the time of wing supination, there is a total range (excluding outliers) of $t/T = 0.3229$ and an interquartile range of $t/T = 0.079$. While the interquartile range of these latencies is quite small, the total range is comparably quite large, meaning that while it is possible that there is a causative relationship between the *RS-PtC* contact and the timing of the end of the downstroke and wing supination, it is not possible for us to conclude this due to the large range of latencies.

Table 6.1: Results of linear regression modelling using the timing of *RS-PtC*, gear engagement (as a categorical variable) and an interaction between these variables to predict the timing of wing supination, the end of the downstroke and the maximum stroke angle reached during the wingbeat. The *F*- and *p*-values are also shown to indicate the significance of each predictor in the linear model, with bold values indicating statistical significance after the application of a false discovery rate algorithm. The total and mean sum of squares, and degrees of freedom for each factor are also shown.

Timing of Wing Supination					
Factor	Sum of Squares	df	Mean Square	<i>F</i> -value	<i>p</i> -Value
<i>RS-PtC</i> Contact Timing	0.318	1	0.318	141.5	<i>p</i> < 0.001
Gear Engaged	0.0141	1	0.0141	6.289	<i>p</i> = 0.012
<i>RS-PtC</i> Contact Timing : Gear Engaged	0.0004	1	0.0004	0.1578	<i>p</i> = 0.691
Error	2.324	1034	0.0022		
Total	2.656	1037			
Timing of Downstroke End					
Factor	Sum of Squares	df	Mean Square	<i>F</i> -value	<i>p</i> -Value
<i>RS-PtC</i> Contact Timing	0.6115	1	0.6115	2252	<i>p</i> < 0.001
Gear Engaged	0.0002	1	0.0002	0.7738	<i>p</i> = 0.379
<i>RS-PtC</i> Contact Timing : Gear Engaged	0.019	1	0.019	69.87	<i>p</i> < 0.001
Error	0.2831	1043	0.0003		
Total	0.9138	1046			
Maximum Stroke Angle					
Factor	Sum of Squares	df	Mean Square	<i>F</i> -value	<i>p</i> -Value
<i>RS-PtC</i> Contact Timing	6395	1	6395	77.03	<i>p</i> < 0.001
Gear Engaged	62139	1	62139	748.4	<i>p</i> < 0.001
<i>RS-PtC</i> Contact Timing : Gear Engaged	8209	1	8209	98.88	<i>p</i> < 0.001
Error	86597	1043	83.03		
Total	163342	1046			

6.4 Discussion

Our high-speed videos reveal that the *PtC* makes extensive contact with the groove in the anterior side of the *RS* towards the end of the downstroke (figure 6.3). As a result the *PtC* is well placed to provide wingbeat-synchronous information about the position of the wing as it reaches the latter half of the downstroke and begins the upstroke. This component could also provide detail regarding the orientation and speed of the wing depending which areas of the *PtC* head are contacted by the *RS*, and how quickly the base is deformed by this contact. Further support for a proprioceptive role of the *PtC* comes from our observations of the *PtC* moving independently of the rest of the hinge on multiple occasions. This would actively change the time at which the two components make contact (supplementary video 6.1), possibly driven by the activity of muscles attached to the basalar sclerite (Miyan and Ewing, 1985a; Nalbach, 1989). Finally, the shape of the *PtC*, with its club-like shape translating large movements at the distal end to small movements at its proximal end, is typical of other sensory organs (Keil, 1997).

Regarding the competing, ‘shock absorber’ hypothesis mentioned by other authors (Chowdhury and Parr, 1981; Wissner, 1988; Nalbach, 1989), we find evidence to the contrary. Plotting the timing of *RS-PtC* contact and the maximum anterior stroke angle reached during the downstroke reveals a significant negative correlation between the two (figure 6.4). If the *PtC* was a shock absorber, we would expect to see a positive correlation: earlier contact between the *RS* and *PtC* would enable the *PtC* to absorb more energy and thereby reduce maximum stroke angle to a greater degree. This is not what we see, from which we infer that *PtC* contact is not related to wing movement in any mechanical capacity.

The *RS* is seen to contact and displace the *PtC* very suddenly as the former disengages from its engagement with either grooves of the *PWP* head as part of the gear change mechanism (e.g. Supplementary video 3.2; Chapters 3, 4 & 5; Pfau (1973); Nalbach (1989); Deora et al. (2015)). During gear 1 or gear 2 wing movements, in which during the downstroke the *RS* makes contact with rear or anterior groove

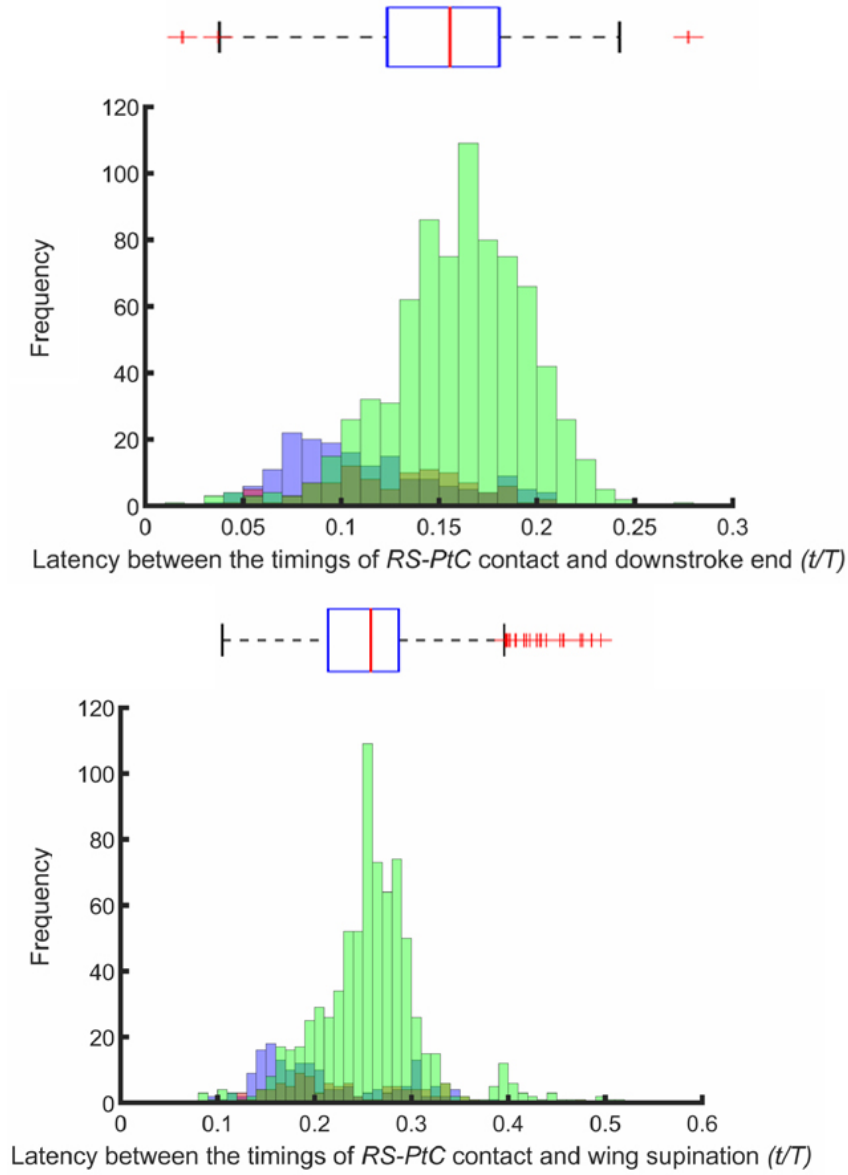


Figure 6.5: Histograms illustrating the variation in latencies between *RS-PtC* contact and the timing of the end of the downstroke (top) or of wing supination (bottom) across differently geared wingbeats. Boxplots above the histograms show the range of the data excluding outliers (black bars), the interquartile range (blue box), the median (red line), and outliers (red crosses) of the whole dataset when not separated by gear. Green bars - gear 3 wingbeats; red bars - gear 2 wingbeats; blue bars - gear 1 wingbeats.

of the *PWP* head, respectively, the *RS* flicks quickly upwards and knocks the *PtC* dorsally in the process. In gear 3 wing movements, however, the *RS* makes much more gradual contact with the *PtC* and there is no sudden movement. *RS-PtC* contact during gear 1 or 2 wingbeats is brief and unlikely to have any mechanical influence on the wing compared to those in gear 3, but the intimate contact between the *RS* and *PtC* and the variation in the extent and speed of engagement across gears could provide adequate sensory information to determine changes in wing motion.

The position of the wing at the point of *RS-PtC* contact also varies significantly across gears (figure 6.3) depending on whether the *RS* and *PWP* physically interact. However, regardless of whether or not this interaction occurs, the *RS* and *PtC* tend to make contact shortly before the wingtip moves anterior of the wing hinge (though some wingbeats show contact after this point). The timing of this contact correlates strongly with both the timing of wing reversal at the end of the downstroke (figure 6.4), as well as the timing of wing supination. However, it is important to note that the latency between *RS-PtC* contact and these events varies significantly (figure 6.5). If the *PtC* is indeed a wingbeat synchronous proprioceptor, as suggested by Miyan & Ewing and others (Miyan and Ewing, 1984, 1985a; Nalbach, 1989; Fayyazuddin and Dickinson, 1996), and *RS-PtC* contact triggers the end of the downstroke or wing supination, we would expect the latency between these events to be more consistent. As the *PtC* makes contact with the *RS* shortly before the wing moves anterior of the wing hinge, it may be possible that the *PtC* could detect the position of the wing at this point and inform the timing of events which take place around this point (such as the end of the downstroke or timing of supination). Due to the latency between *RS-PtC* contact and the events observed here, however, we cannot confidently conclude the the *PtC* is a proprioceptor responsible for determining the time of these events, though it remains possible that it informs other events that require information about the position of the wing, or the speed of its approach, towards the end of the downstroke.

Due to the speed of movement of components in the flight motor, it may not be possible for *PtC* contact to inform events, such as the end of the downstroke, that occur so soon afterwards because of the time required to induce nervous activity. Miyan & Ewing, for example, correlated *PtC* stimulation (here occurring at the end of the downstroke) with action potentials in the nerve innervating the *b1* muscle (Miyan and Ewing, 1984), which becomes active during, or at the end of, the upstroke. This muscle attaches to the basalar sclerite near the wing hinge, and receives an action potential on almost every wingbeat (Heide, 1983; Egelhaaf, 1989; Heide and Götz, 1996; Tu and Dickinson, 1996; Fayyazuddin and Dickinson, 1996, 1999; Balint and Dickinson, 2001). The timing of activation within the wingbeat varies, however, and this influences the movement of the basalar sclerite. Furthermore, due to a ligament connecting this sclerite to the wing, this causes changes to wing kinematics (figure 6.6, Nalbach (1989); Tu and Dickinson (1994)). Miyan & Ewing found that the delay between *PtC* stimulation and *b1* nerve action potential spike was around 2 ms. At the wingbeat frequencies recorded in this study (150.3 ± 20 Hz), the whole wingbeat lasts between 6-8 ms, *PtC* stimulation occurs between 2.73 - 3.50ms after the start of the wingbeat, and the beginning of the upstroke occurs between 3.78 - 4.27ms after the start of the wingbeat. The 2ms delay between *PtC* stimulation and *b1* activity would therefore result in the stimulation of the muscle during the upstroke, roughly when activity has been previously recorded.

Nalbach (Nalbach, 1989) suggests that *PtC* stimulation may be responsible for altering the phase of activation of the *b1* muscle, and our findings here lend weight to this idea. Furthermore, Fayyazuddin & Dickinson (Fayyazuddin and Dickinson, 1996, 1999) identified a strong monosynaptic connection between the afferent nerves from mechanosensors of both the halteres and the wings, with the *b1* motor neuron which is thought to entrain the timing of *b1* activation (Heide, 1983). The *PtC* could therefore be a component within this system that is responsible for helping fine tune the timing of *b1* activation within the wingbeat.

The muscles of the basalar sclerite have also been suggested to be responsible

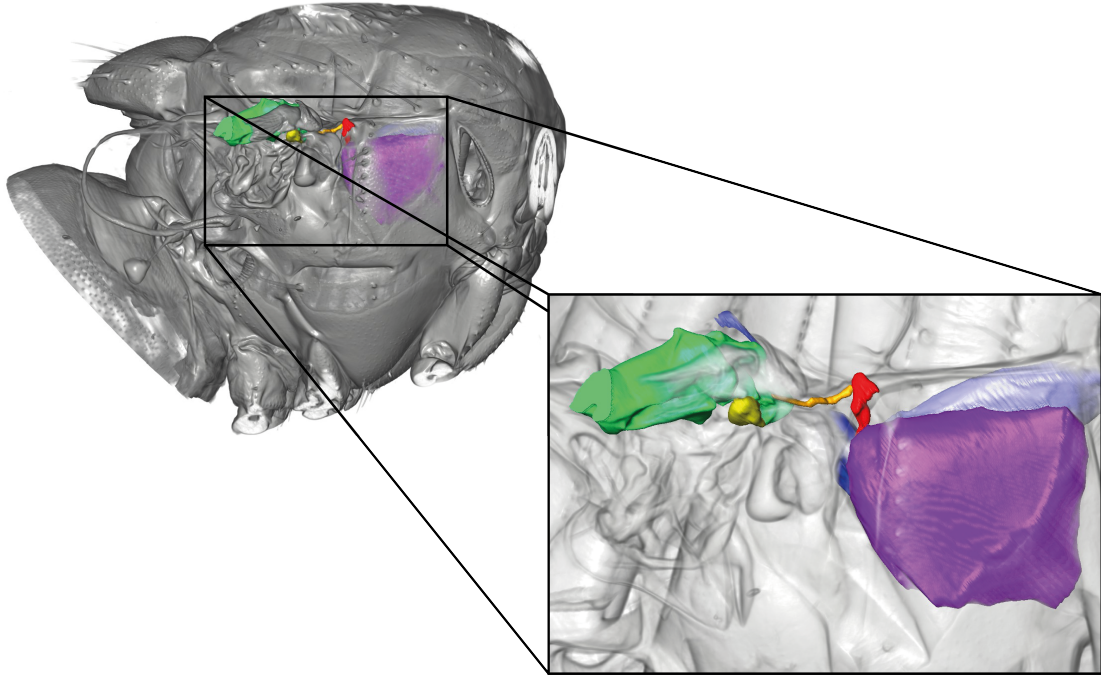


Figure 6.6: A μ CT scan of a *C. vicina* thorax with the Pterale C, basalar sclerite, and basalar muscles highlighted to demonstrate the proximity of these muscles to the *PtC*, which is connected to the same membrane as the basalar sclerite. As a result of this contact through the membrane, movement of the sclerite because of muscular activity may govern the position of the *PtC*. A ligament connects the basalar sclerite to the radial vein of the wing, allowing muscle activity to control wing kinematics. Green - Radial vein; Yellow - *PtC*; Red - Basalar sclerite; Orange - Basalar ligament; Light purple - *b1* muscle; Dark purple - *b2* muscle; Blue - *b3* muscle.

for the movement of the *PtC* independent of the rest of the wing hinge (figure 6.6), just as we observed in this study (supplementary video 6.1; Miyan and Ewing (1984, 1985a); Nalbach (1989)). If this is indeed the case, movement of the *PtC* could be a mechanism through which these insects can induce the muscular responses triggered by early or late *RS-PtC* contact. Investigating this possibility is outside the scope of this work, but the potential relationship between these components deserves further study.

6.5 Conclusions

The role of the *PtC* has long remained inconclusive, but our results presented here suggest that it may be a wingbeat-synchronous proprioceptor. The *RS* and *PtC* make contact during the latter half of the downstroke shortly before the wing moves anterior of the wing hinge. While we found significant relationships between the

timing of *RS-PtC* contact and both the timing of wing reversal at the end of the downstroke, and the timing of supination, there is significant variation in the latency between this contact and these events. While it is possible that the *PtC* does inform these events, and others that may require information about the position of the wing as it nears the end of the downstroke, we cannot state this conclusively. For example, this information about the position of the wing may complement other sensory mechanisms that determine the timing of *b1* muscle activation, which is active during every wingbeat but varies in the precise timing of its activation. This contact is not a shock absorbing mechanism responsible for controlling the forward amplitude of the wing, and indeed we found no evidence of the *PtC* being able to restrict wing movement in any way. Our finding of another possible sensory component within the wing hinge illustrates the importance of proprioception in controlling wing kinematics during flight, and may provide useful inspiration in the design of stabilising sensory systems within micro- or nano-air vehicles.

Appendices

Appendix D

Wing Kinematic Linear Models

Table D.1: Coefficient estimates and their standard errors for variables in the linear equations estimating the timing of wing supination, the timing of the end of the downstroke and maximum stroke angle generated through linear regression modelling. The associated t -statistics and p -value of each predictor variable within the models are also shown.

Timing of Wing Supination				
Predictor	Estimate	Standard Error	t -Statistic	p -Value
Intercept	0.5714	0.0391	14.6	$p < 0.001$
<i>RS-PtC</i>				
Contact Timing	0.2896	0.0784	3.691	$p < 0.001$
Gear Engaged	-0.0115	0.0146	-0.7881	$p = 0.431$
<i>RS-PtC</i>				
Contact Timing :	0.0121	0.0305	0.3972	$p = 0.691$
Gear Engaged				
Timing of Downstroke End				
Predictor	Estimate	Standard Error	t -Statistic	p -Value
Intercept	0.3041	0.012	25.3	$p < 0.001$
<i>RS-PtC</i>				
Contact Timing	0.6146	0.0244	25.22	$p < 0.001$
Gear Engaged	0.0386	0.0046	8.389	$p < 0.001$
<i>RS-PtC</i>				
Contact Timing :	0.0818	0.0098	-8.359	$p < 0.001$
Gear Engaged				
Maximum Stroke Angle				
Predictor	Estimate	Standard Error	t -Statistic	p -Value
Intercept	-58.35	6.646	-8.78	$p < 0.001$
<i>RS-PtC</i>				
Contact Timing	81.17	13.48	6.022	$p < 0.001$
Gear Engaged	36.64	2.548	14.38	$p < 0.001$
<i>RS-PtC</i>				
Contact Timing :	-53.79	5.41	-9.944	$p < 0.001$
Gear Engaged				

Chapter 7

Four dimensional imaging of Dipteran control muscles

The mechanisms by which Dipteran insects control their wing movements are extremely complex, and the flight motor is difficult to study with classical techniques. Here we make use of a modern methodology, time-resolved synchrotron X-ray computed microtomography, to generate four-dimensional images of the thorax of *Calliphora vicina*. By studying the images generated here, we calculated muscle strain patterns of three muscles of particular importance within the flight motor: *I1*, *I2* and *b1*. Through linear modelling techniques, we have correlated bilateral differences in muscle strain patterns with bilateral differences in wing kinematics and identified significant relationships. After averaging these models, we have constructed linear models capable of predicting bilateral differences in wing kinematics using bilateral differences in muscle strain. These models have also enabled us to infer the mechanisms through which these muscles bring about changes in wing movements. We propose that all the muscles studied here act as springs of variable stiffness, restricting and limiting the movement of the sclerites of the wing hinge to which they attach. Several characteristics of the muscles have evolved to optimise their work output, such as their anatomy, timing of activation, and the mechanical advantage under which they act. These principles could be readily applied to the design of

machines on a similar scale to that of insects, such as MAVs.

7.1 Introduction

The field of bio-inspiration, and the researchers working within it, hope to apply the knowledge of mechanisms found in nature to the design of novel and more efficient vehicles and systems. Dipteran insects are able to perform remarkable aerial acrobatics, and do so for prolonged periods of time (Götz, 1987). It is this combination of flight ability and efficiency that has attracted engineers hoping to match these capabilities (Shyy et al., 1999; Ramananarivo et al., 2011; Chin and Lau, 2013; Liu et al., 2017; Chen et al., 2017). However, the many qualities that afford these traits, such as their small size, rapid wingbeat frequencies, and complex internal muscular anatomy, are the same qualities which make these animals difficult to study. As a result we have an incomplete understanding of the Dipteran flight motor, and we are unable to apply the principles found in this system to our own engineering applications, such as the design of MAVs.

The gross movement of the wings during the wingbeat is achieved by the activity of two large groups of muscles within the thorax, the dorsoventral (DVMs) and dorso-longitudinal muscle (DLM). Together, these ‘power muscles’ occupy the vast majority of the muscle mass within the thorax. As the DVMs contract they deform the thorax, compressing it dorsoventrally and stretching the antagonistic DLM (figure 7.1). Both these muscle groups are myogenic and stretch activated, such that when one group is finished contracting the other group is stretched sufficiently to begin contraction itself (Williams and Williams, 1943; Pringle, 1949; Roeder, 1951; Nachtigall and Wilson, 1967; Pringle, 1981; Wisser and Nachtigall, 1991; Dickinson and Tu, 1997; Bartussek et al., 2013). The features of these fibrillar muscles explain why the Diptera, and other Neoptera, can achieve wingbeat frequencies of over 100 Hz (Roeder, 1951; Chan and Dickinson, 1996; Tu and Dickinson, 1996; Bartussek et al., 2013; Ha et al., 2013).

Together, the DLM and DVMs are often referred to as ‘indirect’ flight muscles,

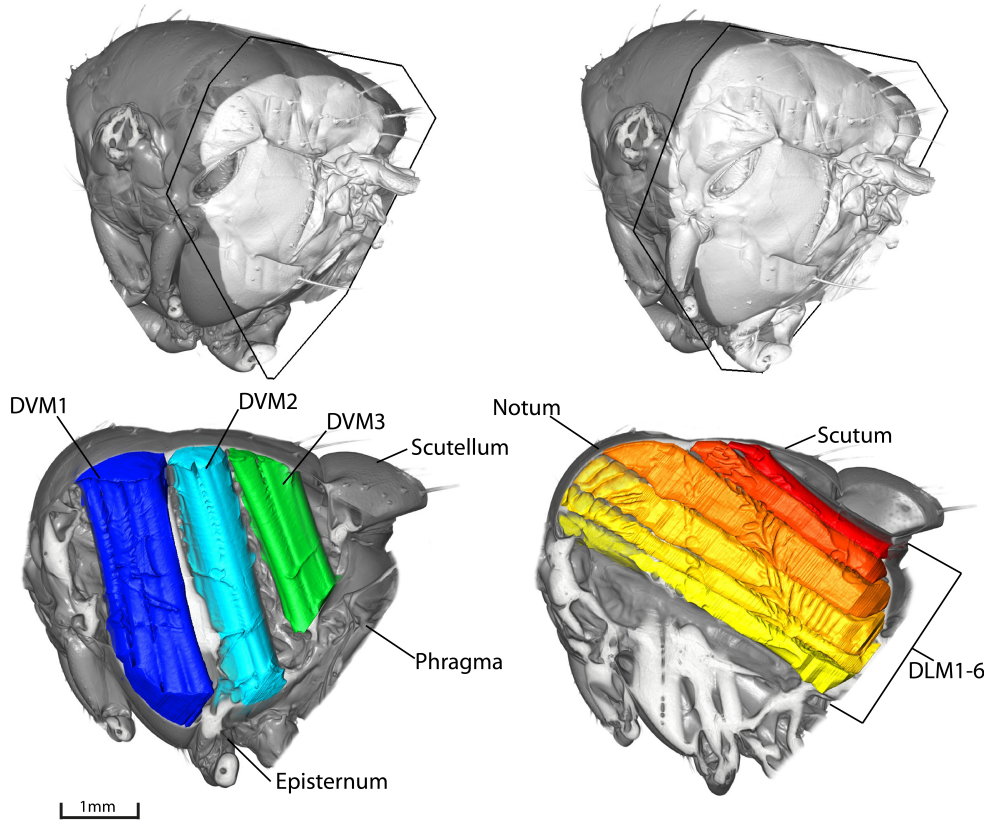


Figure 7.1: μ CT scans of the thorax of *Calliphora vicina*, windowed to reveal the anatomy of the power muscles (colour) and the parts of the thorax to which they attach (grey). Top: Figures illustrating the plane through which the thorax has been windowed (the white area indicates the volume which has been removed). Left: The three dorsoventral muscles (DVMs). Right: The six subunits of the dorsolongitudinal muscle (DLM). Scale bar = 1mm.

as they do not attach directly to the wing or wing hinge. Despite this, by deforming the surrounding exoskeleton, the force these muscles impart is translated into wing movement. Thorax deformation transmits force through a complex series of thoracic components, which in turn causes movement of the components of the wing hinge, thereby moving the wings (Boettiger and Furshpan, 1952; Thomson and Thompson, 1977; Miyan and Ewing, 1985a,b; Bennet-Clark, 1986; Ennos, 1987; Pfau, 1987; Miyan and Ewing, 1988; Brennan et al., 2003; Tang and Brennan, 2011). However, these fibrillar muscles are unable to induce large bilateral asymmetries in the wing-beat. This is because the scutum (to which both sets of power muscles attach) and the scutellum (which transmits scutum deformation to the wing hinge) are rigid structures with no joints separating the left and the right sides. Any muscular force is therefore translated equally to both wings (though see (Lehmann et al., 2013)).

Indeed, if the scutellum is lesioned to separate the left and right sides of the insect, individuals lose this bilateral synchronicity (Deora et al., 2015).

Muscles without bilateral independence are still able to influence wing kinematics by changing the properties of the flight motor, but they must do so symmetrically. The pleurosternal muscles, for example are thought to alter wing kinematics by changing the stiffness of the thorax, while the tergotrochanter is hypothesised to initiate flight and trigger a jump (Nachtigall and Wilson, 1967; Heide, 1968; Kutsch and Hug, 1981; Heide, 1983; Miyan and Ewing, 1985a; Dickinson and Tu, 1997).

The remaining 13 pairs of control muscles, however, are independent between the left and the right sides, and so are capable of causing the bilateral asymmetries required to induce torques and manoeuvres (Williams and Williams (1943); Nachtigall and Wilson (1967); Wisser and Nachtigall (1984), reviewed in Dickinson and Tu (1997)). Many of these muscles attach to exoskeletal components of the wing hinge (sclerites), and are thought to influence its shape through their activity. As the force of the power muscles is transmitted through the wing hinge to the wings themselves, these muscles change the way in which the wing moves. Indeed, electrophysiological studies have confirmed that the activities of these muscles correlate with changes in ipsilateral wing movements (e.g. Nachtigall and Wilson (1967); Heide (1968, 1983); Egelhaaf (1989); Tu and Dickinson (1994); Lehmann and Götz (1996); Heide and Götz (1996); Tu and Dickinson (1996); Fayyazuddin and Dickinson (1999); Balint and Dickinson (2001, 2004); Lindsay et al. (2017)). More recent investigations have also made use of novel techniques, such as real-time calcium imaging to study the activity patterns of these muscles in more detail (Lindsay et al., 2017).

Throughout these studies, two sclerites emerge as being particularly important within the control of wing kinematics, the first axillary sclerite (*Ax1*) and the basalar sclerite. *Ax1* is intimately involved in the wing hinge and the movement of the wing. It articulates to several other sclerites of major importance in the transmission of force from the power muscles through to the wing, such as the parascutal shelf, and *Ax1* also articulates directly with the wing itself (figure 7.2; Boettiger and Furshpan

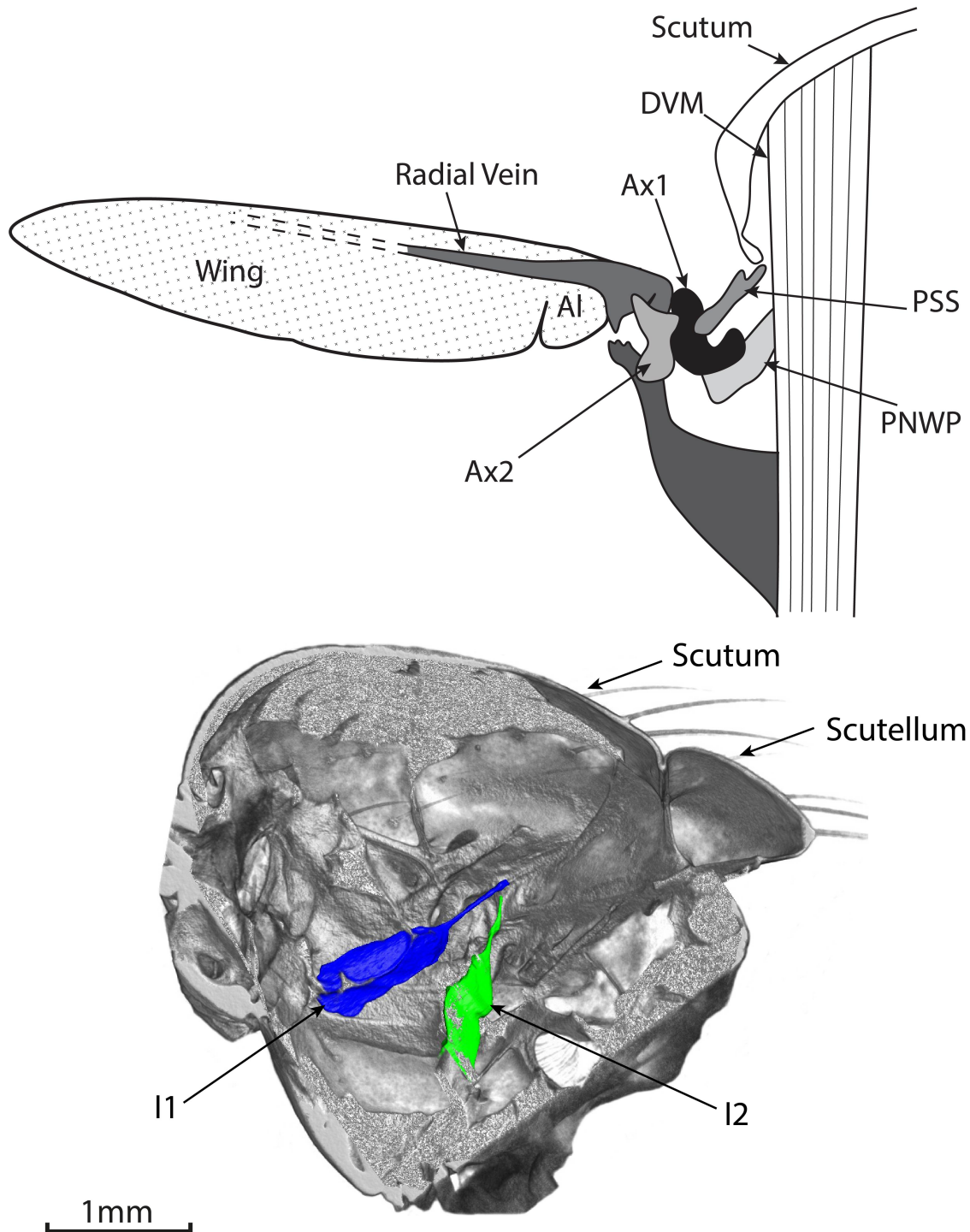


Figure 7.2: The position of *Ax1* and the muscles that attach to it within the thorax and the wing hinge. Top: a transverse cross section of the *C. vicina* wing hinge illustrating the configuration of several of the sclerites that make up the wing hinge. Note in particular the articulation sites of *Ax1* with the *PSS*, *Ax2* and the wing. Adapted from (Walker et al., 2012). Bottom: A μ CT-scan cross section of a *C. vicina* thorax taken along the long axis of the insect illustrating the attachment sites of *I1* (blue) and *I2* (green). Anterior is to the left in this bottom panel. Abbreviations: *Al* - Alula, *Ax1* - First axillary sclerite, *Ax2* - Second axillary sclerite, *DVM* - Dorsal ventral muscles, *PNWP* - Posterior notal wing process, *PSS* - Parascutal shelf. Scale bar = 1mm.

(1952); Thomson and Thompson (1977); Chowdhury and Parr (1981); Miyan and Ewing (1985a, 1988) and reviewed in Deora et al. (2017)). Two muscles attach to *Ax1*, the *I1* and *I2* muscles, both of which have a long tendon connecting the muscle to its attachment site on this X-shaped sclerite. These muscles attach to a very similar site on the sclerite, but originate from opposite sides of the body (figure 7.2). A more detailed description of these muscles can be found in: Nachtigall and Wilson (1967); Chowdhury and Parr (1981); Wisser and Nachtigall (1984) and Dickinson and Tu (1997).

The basalar sclerite, on the other hand, is found anterior to the wing hinge itself, but it is connected to the wing by a tendon. As a result, its movement can influence that of the wing. The triangular head of the sclerite can be seen externally anterior to the wing hinge, and a rod-like extension from the head projects medially into the body, to which two muscles attach: *b1* and *b3*. *b1* attaches anteriorly to the internal rod, having originated further antero-dorsally within the thorax, while *b3* attaches to the opposite side of the rod, and projects postero-dorsally into the thorax via a long tendon. A third, larger muscle, *b2*, connects to the head of the sclerite, and expands in a triangular shape antero-ventrally to its origination point within the thorax (figure 7.3). More detailed descriptions can be found in: Williams and Williams (1943); Wisser and Nachtigall (1984); Miyan and Ewing (1985a); Dickinson and Tu (1997); Deora et al. (2017). Note that we follow the nomenclature of Wisser and Nachtigall (1984).

Previous studies confirm that these control muscles are responsible for altering wing kinematics, but they have not been able to determine the exact mechanisms through which this happens. Indeed, the biomechanics of much of the dipteran flight motor is still not fully understood (Boettiger and Furshpan, 1952; Thomson and Thompson, 1977; Miyan and Ewing, 1985a,b; Bennet-Clark, 1986; Ennos, 1987; Pfau, 1987; Miyan and Ewing, 1988; Wisser and Nachtigall, 1991; Gronenberg, 1996; Brennan et al., 2003; Tang and Brennan, 2011). This is, however, unsurprising given how difficult the system is to study, due to its size (on the scale of micrometres),

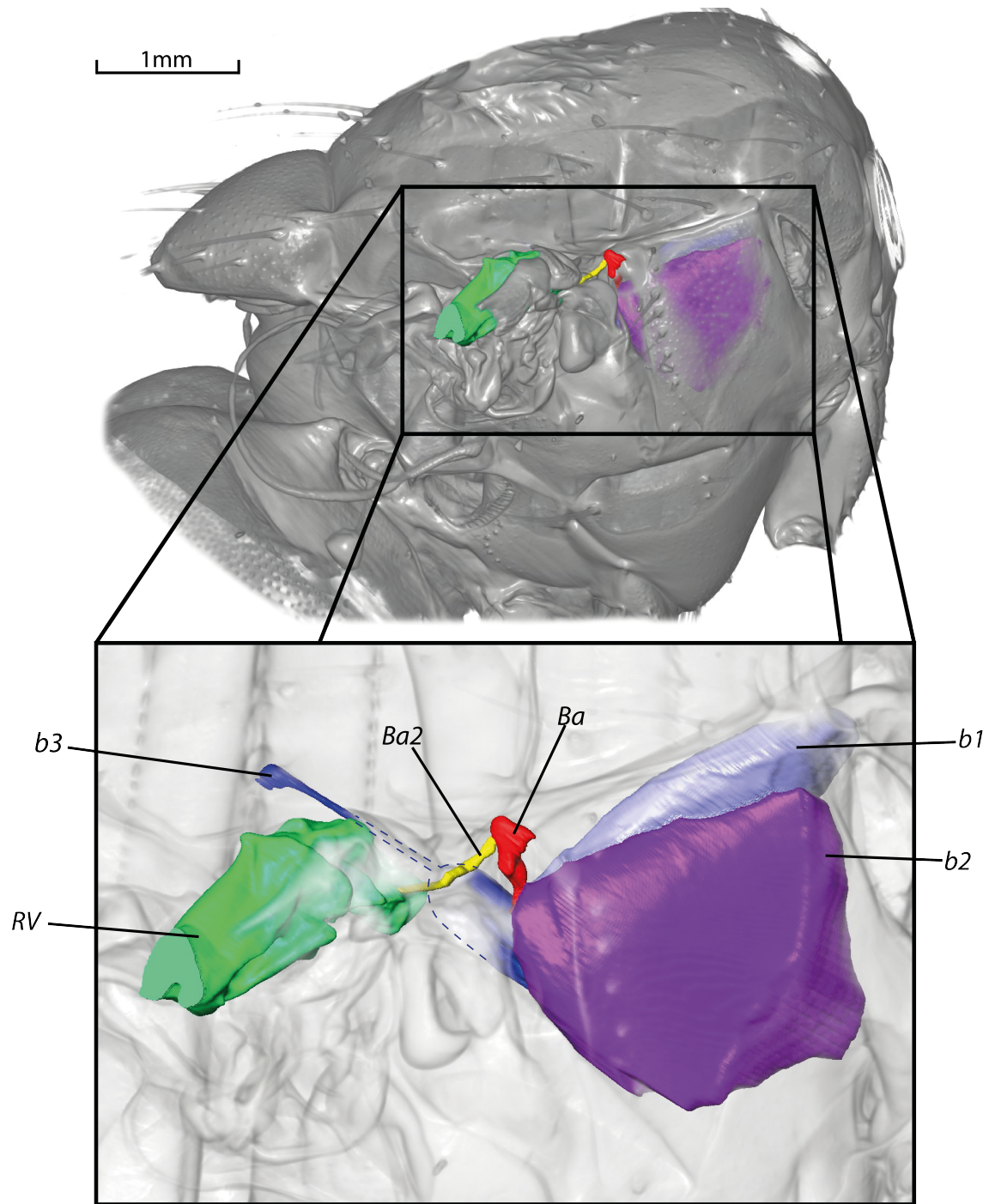


Figure 7.3: A μ CT scan image of the basalar sclerite-muscle complex of *C. vicina*, illustrating the position of the sclerite and the attachment sites of the three basalar muscles. The ligament that connects the basalar sclerite via the basalar 2 is also shown. Abbreviations: *b1-3* - Muscles 1-3 of the basalar sclerite; *Ba* - The basalar sclerite, with its triangular head and medially projecting rod-like body; *Ba2* - The basalar 2 component and ligament connecting to the radial vein; *RV* - Radial vein. Scale bar = 1mm.

rate of movement (over 100 Hz), internalised musculature, and sheer complexity of the system. Gaining an understanding of these mechanisms could prove crucial both in the engineering of mechanisms on a similar scale to that insects, and in implementing effective strategies for flight control in flapping wing vehicles.

Fortunately, modern techniques are enabling us to visualise the dipteran flight motor in previously impossible manners. In 2014, Walker et al. (Walker et al., 2014) utilised time-resolved synchrotron X-ray computed microtomography to construct a four-dimensional image of the blowfly (*Calliphora vicina*) flight motor, revealing hitherto unknown muscle movements. The authors were also able to quantify the strain patterns of several muscles and correlated them to high or low amplitude wing movements, allowing them to gain insights into the biomechanics through which these muscles bring about wing kinematic change.

Here, we extend the use of this method, studying a wider variety of wing kinematics, and focusing on three muscles of particular importance within the flight motor of the *C. vicina* (*I1*, *I2* and *b1*) under significantly greater time resolution across the wingbeat. We quantify bilateral differences in muscle movements and correlate these to bilateral differences in wing kinematics, and find that changes in the movements of each muscle are significantly correlated to wing kinematic asymmetries. Using these new insights, we suggest mechanisms through which this could occur, concluding that the control muscles act primarily through inhibiting the movement of the sclerites to which they attach. Finally, we identify the underlying principles responsible for amplifying the force output of the muscles, which allow them to absorb the kinetic energy output by the power muscles and alter wing kinematics in doing so.

7.2 Materials and Methods

Unless otherwise specified, the methodologies utilised here with regards to insect preparation and radiograph acquisition are the same as those outlined in Appendix A.1, while those relating to camera calibration, and wing kinematic reconstruction

and definitions are the same as those utilised in Chapter 3. This section outlines the techniques unique to this chapter, and refers to other chapters for methodologies which have already been described.

7.2.1 Insect Preparation

We separated the adult insects separated into two groups, one of which we kept on an ambient daylight cycle, while the other group was kept on a 12 hour phase-shifted cycle. This ensured we had a supply of active insects over a 24 hour period of work during data collection.

7.2.2 Stimulus Conditions During Flight

To stimulate flight, we positioned a fan above the insect to which we connected a humidified air stream (figure 2.3). This provided a constant airstream of around 1 ms^{-1} which acted as a stimulus for flight, and also served to cool the insect.

To induce left-right asymmetries in wing movements, we also placed a paper cylinder patterned with graded alternating black and white stripes around the insect. When the insect began rotation during radiograph acquisition, it was thereby presented with a moving stripe pattern rotating across its visual field which induced a compensatory roll response. So as not to impede radiograph acquisition, we removed a 1cm strip from the cylinder at the same height as the insect's thorax and replaced it with polyimide Kapton® tape. This did not significantly affect the quality of the radiographs collected.

7.2.3 Wing Kinematics Measurement

Unlike in previous chapters, we were unable to automatically determine the wing pitch angle, and instead took a sample of 10 wingbeats equally spaced across the recording for each individual. Utilising the previously determined calibration settings, we clicked the midpoint of the leading and trailing edges of each wing in each frame of the wingbeats. By measuring the angle of the line connecting these

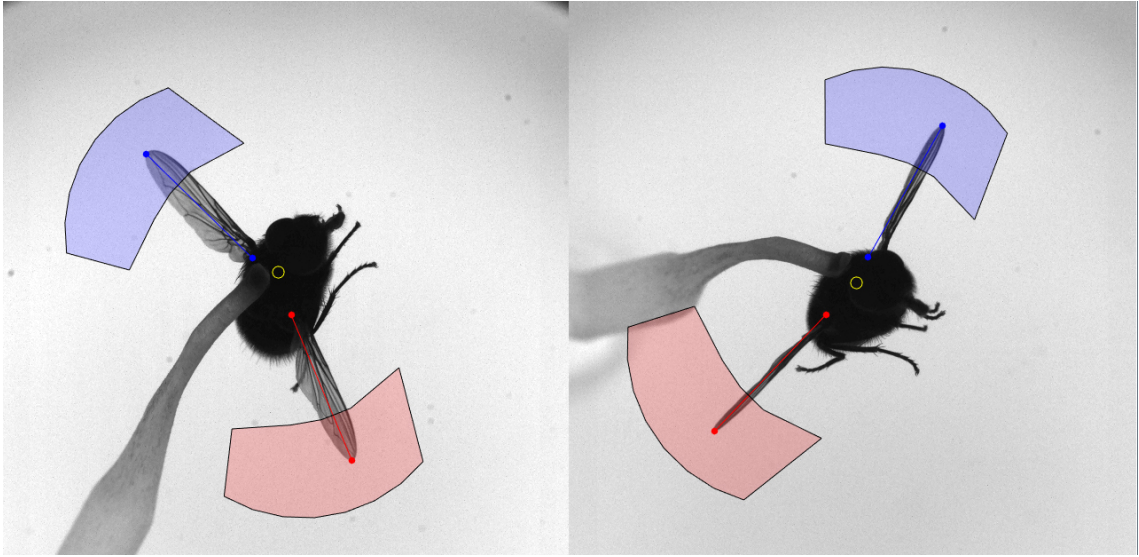


Figure 7.4: Video frames illustrating the collection of three-dimensional wing kinematics using two synchronised cameras. Blue and red dots indicate the left and right wings, respectively, and the lines leading to these points from the wing base are equal to the wing length. The red and blue patches indicate the cropped area of the frame in which the tracking algorithm will search for the wingtip (the farthest point in the outline of the wing obtained through background subtraction) in the next frame. The yellow circle indicates the centre of rotation of the insect during radiograph recording. The plastic rod to which the insect is mounted can also be seen in the both images.

two points relative to the horizontal, we calculated the wing pitch angle across this subset as a representative sample of the whole recording.

7.2.4 Muscle Strain Measurement

We utilised Amira (Thermo Fisher Scientific, Waltham, MA, USA) to visualise the radiographs and identify the muscles of interest: *I1*, *I2* and *b1*. We then manually tracked the movement of the attachment sites of the muscles for each of the twenty individual insects we recorded.

Both *I1* and *I2* possess tendons which are buckled for the majority of the wing-beat, and therefore measuring only attachment sites would underestimate the total length of the muscle-tendon complex. To estimate the total length of the *I1* muscle, we segmented the muscle from the radiographs using Amira then used a custom written MATLAB script to skeletonize the shape of the muscle (Walker et al., 2014). Due to the small width of the *I2* tendon, it was not possible to use the same method to segment it from the radiograph. Instead, because the length of the tendon could be reliably approximated as a series of straight lines between buckling points (fig-

ure 7.5), we manually clicked on these points throughout the wingbeat. We then included the length of the path between these points in the total muscle-tendon unit length estimation. When we refer to either *I1* or *I2* muscles in the text in the remainder of this study, we are referring to the whole muscle-tendon unit.

In MATLAB, we imported the data acquired from Amira and calculated the lengths of the muscles throughout each timestep (including tendon skeletons if appropriate) and the length of the muscle at rest (acquired from the post-flight scan). To calculate percentage strain, we determined the length of the muscle as a percentage of the mean lengths of both the left and right muscles over the course of the wingbeat (i.e. the mean bilateral muscle length).

To these raw strain measurements, we then modelled the data using either a fourier series or a triangular sawtooth wave to the data, depending on which model had the greatest R^2 value. We trained the phase and width of the sawtooth wave using a nonlinear least squares solver to optimise the fit of the wave to the data. For both *I1* ($R_{Fourier}^2 = 0.9 \pm 0.03$, $R_{Sawtooth}^2 = 0.912 \pm 0.03$, and *I2* ($R_{Fourier}^2 = 0.829 \pm 0.08$, $R_{Sawtooth}^2 = 0.8 \pm 0.07$, mean $\pm$ one standard deviation), a sawtooth wave was a significantly better fit to the data ($t_{39} = 3.738$, $p = 0.006$; $t_{39} = 2.727$, $p = 0.01$, respectively; $n_{Sawtooth} = n_{Fourier} = 20$). For the *b1* muscle, neither wave function was a significantly better fit ($R_{Fourier}^2 = 0.855 \pm 0.1$, $R_{Sawtooth}^2 = 0.853 \pm 0.1$; $t_{39} = 0.633$, $p = 0.531$). In this case, we decided to use a Fourier wave to model the data for the rest of our analyses, as this appeared to be the better fit by visually inspecting the data.

Using the properties of these models, we then calculated the amplitude, offset and phase of the strain. We constructed a fourth measure of muscle strain, maximum length, by summing together the offset and amplitude of each muscle, which expresses the maximum muscle length experienced during the wingbeat as a percentage of the mean bilateral muscle length. We then determined the bilateral differences in strain amplitude, offset and phase by subtracting the measurements made on the right side of the body from the left side.

7.2.5 Linear Model Construction and Model Averaging

Using R (The R Foundation), we constructed single-factor linear models to test for significant relationships between any of the bilateral differences in strain and bilateral differences in wing kinematics. For three muscles, four measures of strain (amplitude only, offset only, maximum muscle length, and phase), and fifteen kinematics produced sixty models. Due to this large number of comparisons, we made use of a false discovery rate technique to reduce the likelihood of type I errors.

We were unable to construct more complex models due to the small size of our dataset, and instead utilised a model averaging method to aggregate the large number of models predicting wing kinematics into a single model for each. The goal of this model averaging method is to amalgamate a large number of simple, one or two factor models, into a single model, and to identify the coefficients of each predictor as a weighted average of the significant models.

After grouping all significant (p -value of the model < 0.05) models together by their response variable (wing kinematic), we were able to construct a confidence set of models for each response variable. Using the Akaike Information Criterion (AIC) of each model, we calculated the model's weight using the following equation:

$$w_i = \frac{\exp(-0.5 * \Delta_i)}{\sum_{r=1}^R \exp(-0.5 * \Delta_r)} \quad (7.1)$$

Where Δ_i is the difference between the AIC of the i^{th} model and that of the model with the lowest AIC, Δ_r is the AIC of the r^{th} model, and R is the total number of models in the group (all having a common response variable). Any model with a weight of less than 10% of the greatest weight in the group is excluded, and the remaining models form the confidence set.

From the models within the confidence set for each response variable, we implement the model averaging method to determine the averaged coefficients for each predictor variable. Using the group of models identified as significantly predicting maximum stroke angle (φ_{Max}) as an example, two models remain in the confidence set:

$$\varphi_{Max} = Intercept + \beta_1 I1_{MaxLength} + \beta_2 I2_{MaxLength} + \varepsilon \quad (7.2)$$

$$\varphi_{Max} = Intercept + \beta_1 I2_{MaxLength} + \varepsilon \quad (7.3)$$

Where β_i is the coefficient of the i^{th} term, and ε is the residual error of the model.

Here, the predictive factors identified as members of significant models are $I1_{MaxLength}$, $I2_{MaxLength}$ and the *Intercept*. These three factors will each form a predictive factor in the weighted average model of φ_{Max} . To identify the weighted average coefficients of each factor, we now group the models based on the presence of each factor. The *Intercept* group for example, would include both models, as it is present in both, while the $I1_{MaxLength}$ group would include only equation (2). For each model within each group, we calculate Δ which is given by the absolute difference between the Akaike Information Criterion (AIC) of that model, and that of the ‘best’ model (possessing the lowest AIC value) in that group. From this, we calculate the exponentiated AIC value of each model, as given by:

$$\exp AIC = \exp(-0.5 * \Delta) \quad (7.4)$$

Each model within each group is then given a new AIC weight by dividing the model’s exponentiated AIC value by the sum of all those in the group. A weighted coefficient estimate for the predictive factor is then calculated by summing together the product of the new AIC weights and the raw coefficient estimates obtained from the original linear model.

In calculating confidence intervals for each coefficient estimate, we incorporate the standard errors of the coefficients in each model, and also take into account that these standard errors are conditional on the model itself, the model selection variance (MSV). The MSV of a given model is the square of the difference between the weighted average coefficient estimate calculated earlier and the raw coefficient

estimate in each model in which it is included. To calculate the unconditional standard errors, we then add together the MSV of the model and the conditional variance (the standard error squared) of the coefficient estimate in that model, then take the square of this sum. The weighted unconditional standard error of each coefficient in each model is given by the unconditional standard error multiplied by the new AIC weight of the model. The sum of these values across the different models gives the weighted unconditional standard error of the coefficient estimate.

Upper and lower confidence intervals can then be calculated by taking the weighted average coefficient estimates and adding or subtracting, respectively, the product of the weighted unconditional standard error with the desired t -value. For a sample of the size in this study ($n = 20$), the t -value for a 95% confidence interval is 1.95.

7.3 Results

7.3.1 I1 and I2 Muscle Anatomy and Kinematics

The μ CT reconstructions collected during this study exhibit the unusual anatomy of $I1$, with its tendon which is shown to be buckled for a large proportion of the wingbeat (previously shown in (Walker et al., 2014)). This investigation has also shown that the long tendon found on $I2$ buckles in a similar manner. The tendon of $I1$ is seen to consistently buckle in roughly the same place in each individual (figure 7.5, supplementary video 7.1). This could indicate the presence of connective tissue which may be responsible for holding the tendon in place, similar to the relationship between the propatagial ligament and the propatagial strut within the propatagium of birds (Brown et al., 1994). The $I2$ tendon buckles too, but in a manner more akin to folding than bending when compared to $I1$ (figure 7.5). It buckles in two places, one at the postero-medial corner of the $Ax1$ sclerite, and another close to where the tendon connects to the muscle itself.

The buckling of $I1-2$ may also explain why we find that a sawtooth wave fit the strain patterns of the muscles better than the Fourier curve that we fit to $b1$ (figure

7.6). The spiked strain pattern shows that as the tendon becomes fully outstretched the strain reaches a peak (seen as the tip of the sawtooth wave), before the muscle begins to shorten rapidly again in a linear fashion due to the tendon buckling.

Studying the reconstructions in more detail, the *I1* tendon becomes outstretched, on average, at around a third of the way through the wingbeat cycle ($t/T = 0.331 \pm 0.04$; mean $\pm$ one standard deviation, t is time and T is the total duration of the wingbeat, where t is equal to zero at the start of the downstroke), and buckles again halfway through at the end of the downstroke ($t/T = 0.501 \pm 0.03$). While the *I2* tendon becomes outstretched at $t/T = 0.325 \pm 0.03$ and buckles at $t/T = 0.475 \pm 0.03$. As a result, both *I1* and *I2* are only able to lengthen and absorb energy for a very small portion of the wingbeat, and so can only influence wing kinematics around the timing of the end of the downstroke ($t/T = 0.5 \pm 0.02$) and the timing of wing supination ($t/T = 0.432 \pm 0.05$).

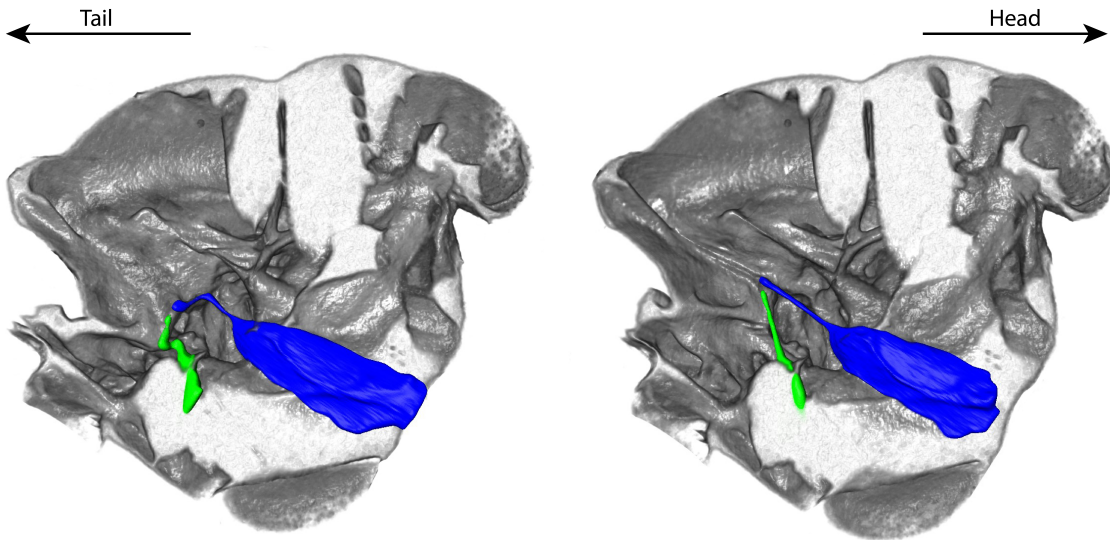


Figure 7.5: A cross section of the thorax displaying buckling in the two muscles of *Ax1*: *I1* (blue) and *I2* (green). The remaining grey area comprises the cuticle and other muscles within the thorax. Left: A buckled configuration at the start of the downstroke. Right: An outstretched configuration at the end of the downstroke.

7.3.2 I1 and I2 Muscle Kinematics - Wing Kinematic Relationships

The anatomies of *I1* and *I2* limit the range of wing kinematics that they are able to influence, as such we would expect only wing kinematics that occur during the period when the muscle tendons are outstretched. Most of the measured kinematics involve some component which occurs during the period when the tendons are outstretched, with the notable exception of minimum stroke angle, which occurs at the end of the upstroke. Table 7.1 indicates the significant relationship found between bilateral differences in *I1-2* activity and bilateral differences in wing kinematics. The findings here show that *I1-2* bilateral differences are significantly correlated with the majority of wing kinematics, though these relationships are more prevalent amongst kinematics involving stroke angle (maximum stroke angle and stroke amplitude) and deviation angle, with fewer correlations found amongst wing pitch angle kinematics.

Significant relationships are found between wing kinematics and every aspect of *I1* strain except for muscle strain phase (table 7.1, Appendix E). This implies that *I1* can only influence wing kinematics through changes in its length, which is itself if brought about by muscular contraction, and not the timing of this contraction during the wingbeat.

Similarly, *I2* shows no significant relationships between bilateral differences in its strain phase pattern. Instead, there are significant relationships between bilateral differences in muscle strain offset and maximum length. These relationships are found only in kinematics which are to some extent determined by the path taken by the wing during the period where *I2* is outstretched, namely stroke amplitude, maximum stroke angle and minimum upstroke pitch angle.

Table 7.1: A summary of the significant correlations (and their sign) between bilateral muscle kinematic differences and bilateral wing kinematic differences. φ = stroke angle (so φ_{range} is equal to stroke amplitude), ϑ = deviation angle, ψ = stroke plane angle, α = wing pitch angle, and t_{sup} is the relative timing of wing supination during the wingbeat. A blank cell indicates no significant correlation. More detailed results can be found in Appendix E. *Note that as stroke angle becomes more negative it indicates the wing travelling further anteriorly.

Muscle Kinematic	φ_{Range}	φ_{Mean}	φ_{Min}	φ_{Max}	ϑ_{Mean}	ϑ_{Min}	ϑ_{Max}	ψ_{Mean}
I1	Amplitude	-	-	-	-	-	neg	-
	Offset	-	-	-	-	neg	neg	pos
	Max Length	pos	-	-	neg*	neg	neg	pos
	Phase	-	-	-	-	-	-	-
I2	Amplitude	-	-	-	-	-	-	-
	Offset	-	-	-	-	-	-	-
	Max Length	pos	-	-	neg*	-	-	-
	Phase	-	-	-	-	-	-	-
b1	Amplitude	pos	-	pos	-	neg	-	pos
	Offset	-	-	-	-	-	-	neg
	Max Length	-	-	-	-	-	-	-
	Phase	-	-	-	-	neg	-	pos

Muscle Kinematic	t_{sup}	Downstroke α_{mean}	Downstroke α_{min}	Downstroke α_{max}	Upstroke α_{mean}	Upstroke α_{min}	Upstroke α_{max}
I1	Amplitude	-	-	-	-	-	-
	Offset	-	pos	-	-	neg	-
	Max Length	neg	pos	-	-	-	-
	Phase	-	-	-	-	-	-
I2	Amplitude	-	-	-	-	pos	-
	Offset	-	-	-	-	-	-
	Max Length	-	-	-	-	-	-
	Phase	-	-	-	-	-	-
b1	Amplitude	-	neg	pos	-	-	-
	Offset	pos	pos	neg	-	neg	-
	Max Length	-	neg	neg	-	neg	-
	Phase	neg	pos	pos	-	pos	-

7.3.3 *b1* Muscle Kinematic - Wing Kinematic Relationships

b1 has a very different anatomy compared to *I1-2*, most notably in that it lacks any conspicuous tendon. As a result, unlike *I1-2*, *b1* has the capability of performing work and influencing the movement of the basalar sclerite (to which it attaches) at any point in the wingbeat if it were to contract. Accordingly, *b1* is the only muscle studied here to show significant relationships between bilateral differences in its phase of strain and bilateral differences in wing kinematics (table 7.1, Appendix E). The strain oscillations of *b1* are also around 180° out of phase compared to *I1-2*, with strain peaking around the time of stroke reversal at the end of the upstroke. This observation is in accordance with *b1* strain amplitude being the only significant factor in the model predicting minimum stroke angle, which occurs around the end of the upstroke.

Most notably, *b1* is found to be a significant predictor in every pitch angle model excluding maximum upstroke pitch angle (for which there were no significant predictors) as well as in predicting the timing of wing supination. These predictors include every measured *b1* strain component (muscle strain amplitude, offset, maximum length and strain phase), which suggests that *b1* and the basalar sclerite to which it attaches are heavily involved in determining the pitch angle of the wing.

7.4 Discussion

7.4.1 *I1*, *I2* and *Ax1*

During the wingbeat, *Ax1* rotates dorsoventrally about its attachment point atop *Ax2* (figures 7.2 and 7.7). The *I1* and *I2* muscles are attached to the medial side of this X-shaped sclerite, while the base of the wing is connected to the lateral side. This configuration means that as the lateral side of *Ax1* rotates ventrally during the downstroke, the wing also moves ventrally while the *I1* and *I2* muscles attached to the medial side of the sclerite are moved dorsally and becomes outstretched. From this observation, *I1* and *I2* could work to limit the dorsoventral rotation of *Ax1*

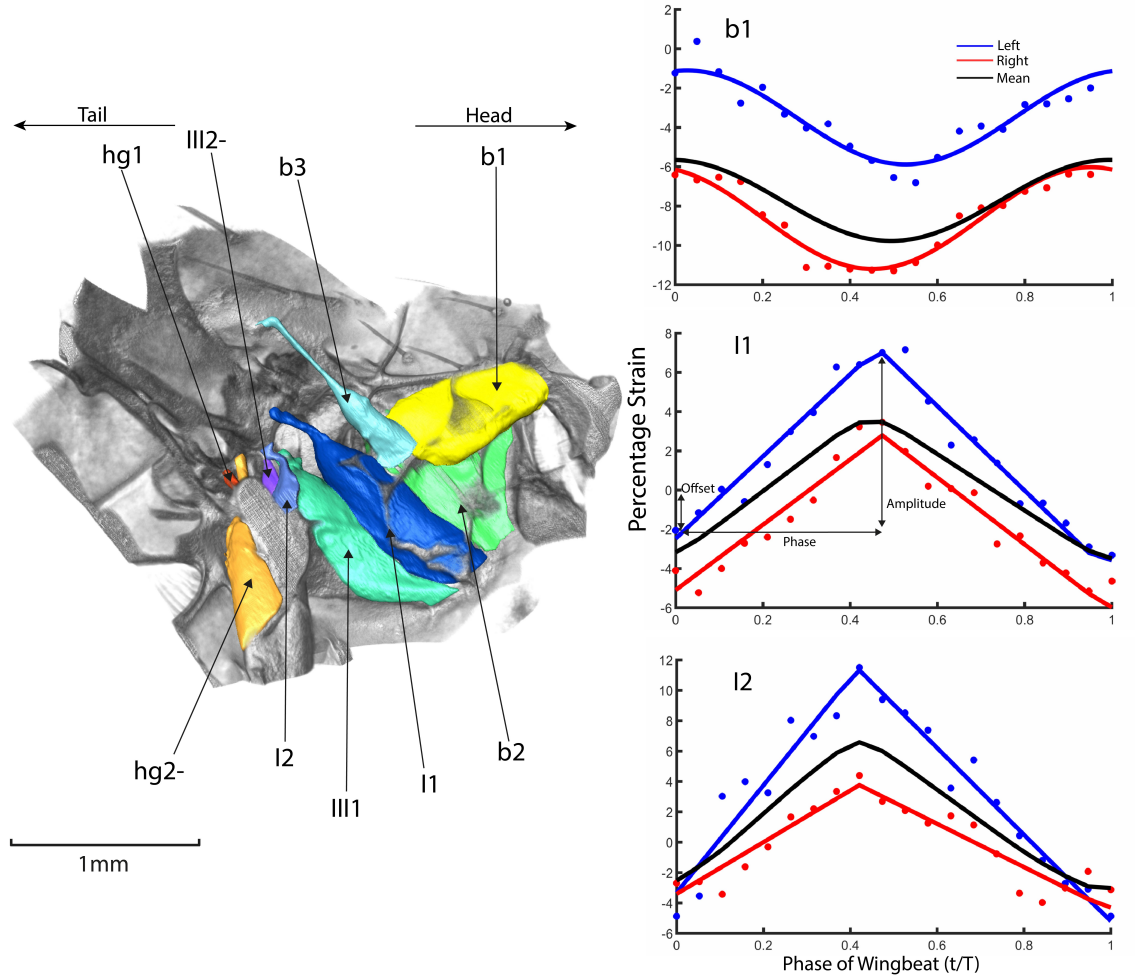


Figure 7.6: A reconstruction of the control muscle complex and strain patterns of the three studied muscles. Left: A μ CT reconstruction of a *C. vicina* thorax cross section showing the control muscle complex of the left wing. Different muscles have been highlighted in various colours. Note the large tendons on *I1-2*, and *b3*. Right: Examples of the percentage strain patterns across the wingbeat for *I1-2* and *b1* in both left (blue) and right (red) muscles, graphically illustrating the strain amplitude, offset and phase. The black lines indicate the mean strain recordings across the wingbeat taken across all individuals. Abbreviations: *b1-3* - muscles 1-3 of the basalar sclerite; *I1-2* - muscles 1-2 of first axillary sclerite; *III1* - muscle 1 of the third axillary sclerite; *III2-4* - muscles 2-4 of the third axillary sclerite; *hg1* - muscle 1 of the posterior notal wing process; *hg2-4* - muscles 2-4 of the posterior notal wing process. Scale bar = 1mm.

during the downstroke.

The results obtained from the averaged linear models suggest that *I1* and *I2* are indeed more influential during the downstroke. Three models in particular, those predicting maximum stroke angle, minimum stroke angle, and stroke amplitude, are very informative regarding the prospective mechanism of muscle action. Maximum stroke angle, which occurs at around the end of the downstroke when both *I1-2* become outstretched (a more negative stroke angle is a greater anterior wing

movement), is predicted by the maximum lengths of *I1-2*. Crucially, the coefficients of these parameters are negative, and here we interpret this to mean that a greater muscle length permits a greater anterior wing movement. As a corollary, a smaller muscle length (which could occur with muscle contraction) is correlated with a smaller anterior wing movement during the downstroke. That neither *I1* nor *I2* significantly predict minimum stroke angle (which occurs at the end of the upstroke when both muscles are buckled) confirms that these muscles are unable to influence sclerite or wing movement at this point.

These observations imply that *I1-2* act by restricting the dorsoventral rotation of *Ax1* about its connection with *Ax2* (figure 7.7). The length of the muscles during the wingbeat determines the limit of *Ax1* rotation. Through this, *I1-2* impart control over stroke angle through doing negative work, absorbing the kinetic energy imparted by the power muscles through the wing hinge via *Ax1*, and in doing so altering the wing kinematics.

It is unclear why the maximum lengths of *I1-2* were the only kinematics found to be significant, when this kinematic itself is composed of the muscle strain amplitude and offset, though it is perhaps due to the small sample size in this study. It is possible that both muscle strain amplitude and offset are correlated to maximum stroke angle, but that this analysis was not powerful enough to identify this. Instead, only a compound kinematic such as maximum muscle length (being equal to the sum of muscle strain amplitude and offset) could be identified as significantly correlated.

Table 7.1 shows that *I1* and *I2* strain patterns have been found to be a significant predictor of other kinematics, all of which are to some extent determined by the movement of the wing during the period in which both the muscles are able to perform work (i.e. when the tendons are pulled taut and the muscles under tension). These kinematics include stroke, deviation, and wing pitch angle. Where *I1-2* may restrict dorsoventral rotation of *Ax1* to control stroke angle, differential restriction of *Ax1*'s antero-posterior rotation may also allow control over wing pitch angle or deviation angle. As *I1* and *I2* attach to opposite sides of *Ax1*, different patterns

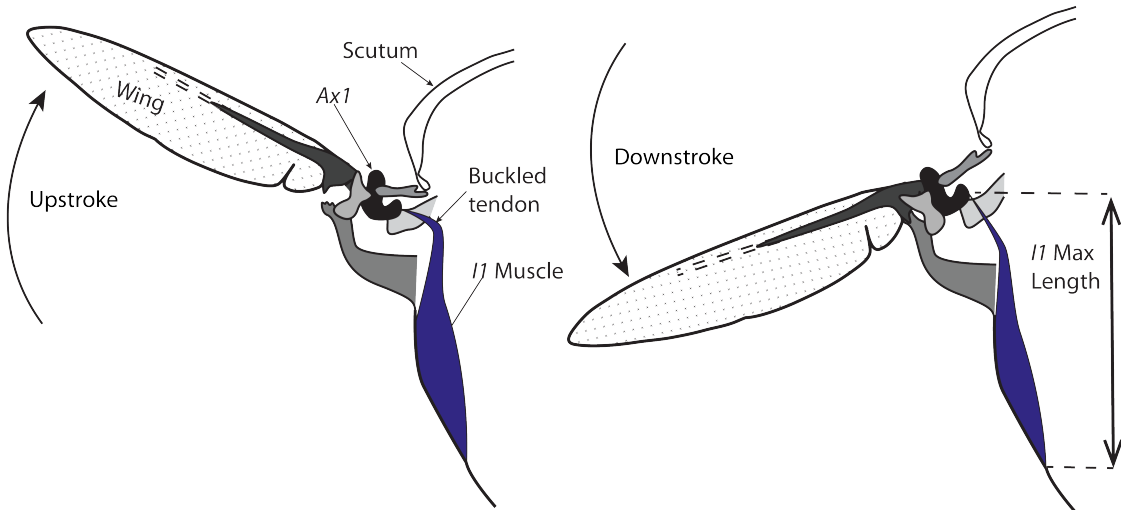


Figure 7.7: An illustration of the proposed mechanism through which *I1* acts to influence wing movement. The rotation of *Ax1* during the wingbeat is restricted at the end of the downstroke by the maximum length of *I1*. The mechanism through which *I2* acts likely works through a similar principle.

of activity in the two muscles could determine the limits of *Ax1* antero-posterior rotation. We did observe differences between the timings of *I1* and *I2* tendon outstretching and buckling, suggesting that *I1* and *I2* could begin to perform work at different times during the wingbeat, thereby influencing *Ax1* rotation.

The ability of *I1-2* to bring about ipsilateral turns by influencing kinematics such as stroke angle, deviation angle, wing pitch angle and stroke plane angle has been identified in previous studies (Heide, 1975; Egelhaaf, 1989; Heide, 1983; Heide and Götz, 1996; Balint and Dickinson, 2001, 2004; Walker et al., 2014; Lindsay et al., 2017). That these findings have been corroborated here reaffirms the ability of time-resolved synchrotron X-ray computed microtomography in studying the role of muscles in insects during flight.

7.4.2 *b1* and the basalar sclerite

The mechanism through which *b1* influences wing kinematics through its attachment to the basalar sclerite is somewhat less clear than that of the *Ax1* complex. It has been previously established that there is a tendon that connects the basalar sclerite via a second component, the basalar 2, to the wing's radial vein (figure 7.3) (Nalbach, 1989; Tu and Dickinson, 1996). Through this, movement of the basalar

lever is capable of influencing wing motion. Our analyses have shown that *b1* strain is a significant predictor of the majority of wing kinematics, though a few notable equations help to elucidate the possible mechanism of action.

Strain amplitude of *b1*, for example, is the sole significant factor in the linear model predicting minimum stroke angle. Minimum stroke angle occurs at the end of the upstroke, around the point at which *b1* reaches its peak strain (figure 7.6), and thereby the time when *b1* can impart maximal force. The coefficient of *b1* strain amplitude in this model is positive (see 7.1; Appendix E), implying that as the amplitude of the muscle strain becomes larger, the degree of posterior movement of the wing at the end of the upstroke also becomes greater.

A greater muscle strain amplitude is likely the result of changing muscle stiffness, which could be the result of a reduced level of muscle contraction, or a change in the phase of muscle activity. That a smaller muscle amplitude would reduce minimum stroke angle, suggests that *b1* restricts the movement of the wing at this point in the upstroke in a similar manner to the way in which *I1-2* control it at the end of the downstroke.

This idea is further reinforced by examining the model predicting stroke amplitude, which is significantly predicted by the maximum length of *I1-2* and *b1*, which themselves predict the maximum and minimum stroke angles. The range between these two angles defines the stroke amplitude, and so the fact that the same predictors of these kinematics also predict stroke amplitude reaffirms both theories of muscle action.

Unlike *I1-2*, *b1* is the only muscle studied here to show significant relationships between strain phase and wing kinematics. We see *b1* strain phase present in the predictive models for every model related to downstroke pitch angle, minimum upstroke pitch angle, the timing of wing supination, as well as both mean and minimum stroke deviation angle (table 7.1). *b1* has been implicated in the modulation of wing pitch angle and supination previously (Dickinson et al., 1993), and others have found that advances in the phase of *b1* activity during the wingbeat is correlated to an

earlier timing of wing supination within the wingbeat, decreases in deviation angle, and an increase in stroke amplitude (Tu and Dickinson, 1996; Heide and Götz, 1996; Balint and Dickinson, 2001; Walker et al., 2014; Lindsay et al., 2017). Each of these kinematic changes corroborate the findings that decreases in *b1* spike activity on one side of the body occurs during ipsilateral turns (Heide, 1975, 1983).

This finding of phase dependent effects on wing kinematics conforms both with the anatomy of *b1*, in that it lacks a buckling tendon, and with previous studies that have identified that *b1* is active on almost every wingbeat (Heide (1968); Ewing (1977); Heide (1983); Tu and Dickinson (1994, 1996); Heide and Götz (1996); Fayyazuddin and Dickinson (1996, 1999); Balint and Dickinson (2001); Dickinson (2005); Lindsay et al. (2017), though see Egelhaaf (1989)). These previous authors have suggested that *b1* is adapted to make small adjustments to wing kinematics on a wingbeat-by-wingbeat basis through changes in the timing of activity. In the case of this study, these changes are evident from bilateral differences in the phase of muscle strain, and the significant relationships between these and wing kinematics.

Few previous authors studying *b1* activity have offered biomechanical explanations for how these changes in muscle activity observed in previous studies bring about changes in wing kinematics. Both Tu & Dickinson (Tu and Dickinson, 1996) and Lindsay et al. (Lindsay et al., 2017) suggest that *b1* can increase stroke amplitude by shifting the position of the wingtip at the end of the downstroke anteriorly (a greater maximum stroke angle in this study). However we suggest that it instead determines stroke amplitude by controlling the position of the wingtip at the end of the *upstroke* (i.e. altering minimum stroke angle).

We believe that this latter hypothesis is more intuitive from a biomechanical perspective. The strain patterns calculated in this study here indicate that *b1* reaches a peak strain at the end of the upstroke, and as such it is in a better position to perform its purported role of negative work (Tu and Dickinson, 1994, 1996) during the upstroke than at the end of the downstroke, as these other authors suggest. Indeed, as *b1* muscle strain decreases during the downstroke (figure 7.6), this would

be a poor opportunity to perform negative work, and *b1* action potentials have been recorded occurring during the upstroke (Heide, 1983; Egelhaaf, 1989; Heide and Götz, 1996; Tu and Dickinson, 1996; Fayyazuddin and Dickinson, 1996, 1999; Balint and Dickinson, 2001). Furthermore, the end of the upstroke would be the point at which the tendon connecting the basalar sclerite to the wing's radial vein would be stretched to its greatest degree and therefore the most effective time to impart force.

The mechanism by which *b1* might control deviation angle, wing pitch angle or wing supination, however is less clear. The path that *b1* takes during the wingbeat differs between wingbeats, and as does the path of the basalar sclerite (Walker et al., 2014). It is possible that these differences are responsible for the changes observed. Strain phase is a consistent component of the predictive models for pitch angle and deviation angle, and seems a likely candidate in an explanation. Changes in muscle phase could determine the path of the muscle and the sclerite. In changing the path of the basalar sclerite, the force vector imparted to the radial vein from the basalar 2 ligament will change in direction, and this could be responsible for altering the orientation of the wing about its long axis and thereby changing the pitch angle. Alternatively, the basalar sclerite may also pull the base of the wing ventrally thereby changing the deviation angle of the wing (figure 7.3).

Several previous authors have identified the potential role of interaction between different muscle groups and sclerite movements in determining wing kinematics (Balint and Dickinson, 2001). Our study here is limited in that it investigated only three of the seventeen control muscles, and the small sample size precludes our ability to build complex linear models with more than a couple of parameters. Indeed, the interactions between the activity of the basalar muscles may explain why some findings here relating to the activity of *b1* do not agree with those of Walker et al.'s study, which utilised similar methods (Walker et al., 2014). Namely, the previous authors' finding that high muscle strain amplitude in *b1* correlates with low wingbeat amplitude, where we have found the opposite correlation here. We also

make use of a larger dataset here, and examine a wider variety of wing kinematics than Walker et al., so it is possible that undersampling in this previous study lead to different conclusions than those found in our current study. Future studies will make use of larger datasets, and examine more muscles, and in doing so will be able to identify the functional mechanisms of other groups and the interactions between them.

7.4.3 Principles of Muscle Action: Anatomy, Activity, and Advantage

Many of the significant relationships we have identified between muscle strain and wing kinematics relate to the maximum length of the muscles during the wingbeat. This suggests that these muscles may act to limit the movement of wing hinge components (and therefore the wing too) by resisting further lengthening and perform negative work. It is understood that muscles are able to produce a greater force, and do so more economically, while performing negative work compared to positive work (see Chapter 2 section , Fenn (1924); Abbott et al. (1951, 1952); Bigland-Ritchie and Woods (1976); Lindstedt et al. (2001); Elmer and LaStayo (2014)). Maximising energy absorption is crucial for the control muscles, as they must act against the force produced by the much larger power muscles. Our study identifies the restriction of sclerite motion, possibly through negative work, as one mechanism by which the dipteran flight motor may accomplish this, and it also highlights other principles of muscular force amplification: anatomy, activation timing, and mechanical advantage.

b1 is specialised in its anatomy, in that its relatively large cross section increases its ability to produce force and do negative work (Ikai and Fukunaga, 1968; Maughan et al., 1983; Jones et al., 2008). As well as this, its lack of a large tendon enables it to do work on the basalar sclerite at all times during the wingbeat, thereby allowing the muscle to influence wide variety of wing kinematics.

The muscles of *Ax1*, on the other hand, have distinctive anatomies in which a

large portion of the muscle length is occupied by a tendon which itself is buckled for the majority of the wingbeat. This anatomy serves several purposes for the function of these muscles. Firstly, it permits the large range of movement of *Ax1*, and thereby enables the wing to move without any impedance that might occur if the muscle was constantly under strain.

Secondly, it absolves the need for the nerves which innervate these muscles to generate a precisely timed action potential on every wingbeat, which is presumably energetically expensive. Instead, these muscles can remain contracted and still only influence the wingbeat towards the end of the downstroke when their input is required to control the stroke angle. The degree of contraction can presumably be altered through whether or not action potentials reach the muscle on consecutive wingbeats (Heide, 1975, 1983; Heide and Götz, 1996; Balint and Dickinson, 2004), but there is no requirement for these to occur on every wingbeat or to be timed precisely. The unique buckling tendons of *I1-2* therefore appear to be features which save the muscles, and the flight motor as a whole, substantial amounts of energy.

The attachment site of *I1-2* to *Ax1* are also adapted to optimise the mechanical advantage of these muscles. As mentioned earlier, *Ax1* is intimately involved in the wing hinge and articulates directly with the wing. The length of the muscles compared to the wing, and its attachment sites relative to the rotation axis of *Ax1* results in an effective mechanical advantage of 0.163 and 0.14 for *I1* and *I2*, respectively. This configuration means that the small restrictions imparted by *I1-2* on *Ax1* rotation about its articulation with *Ax2* (figure 7.7) result in proportionally large changes in the movement of the wing. This comes at the cost of requiring a large force from the muscles due to the small mechanical advantage, but this is somewhat mitigated by the utilisation of negative rather than positive work as discussed earlier. Similarly, the small movements of *b1* are amplified into larger movements of the wing by the connection of the basalar sclerite to the base of the radial vein by a small tendon. Through this, these muscles are able to amplify their small movements into large changes in wing motion.

The effect of this amplification also depends on the point in the wingbeat when the muscles are doing work. Previous work by other authors has confirmed that *b1* can alter wing kinematics differently depending on when during the wingbeat it receives an action potential (Heide and Götz, 1996; Balint and Dickinson, 2001). As the various components of the wing hinge translate and rotate during the wingbeat, any input into the system through muscular force will have different effects depending on the precise configuration at that moment in time. The ability of the muscles to do work will also change throughout the wingbeat. *b1* is at its shortest at the end of the downstroke, and longest at the end of the upstroke, so by becoming active early on in the downstroke there would be greater scope to restrict the movement of the basalar sclerite (Brown and Donnelly, 2011).

7.5 Conclusions

Time-resolved synchrotron X-ray computed microtomography has now been utilised in two separate studies to examine the biomechanics of the dipteran flight motor (Walker et al., 2014). This study expands the use of this technique, and has also utilised linear model averaging to determine significant relationships between muscle strain patterns and wing kinematics. We have also constructed models to predict the movements of the wings based on muscle strains, and determined the mechanisms through which these muscles act to influence wing movement.

We suggest that all three of the muscles studied here act as variable tension springs to restrict the motion of the sclerites to which they attach. *I1-2* and *b1* can therefore influence the stroke angle of the wings at the end of the downstroke and upstroke, by limiting the motion of *Ax1* and *b1*, respectively. The mechanisms through which these muscles influence other kinematics is less clear, though it may be related to the rotation of *Ax1* by *I1-2* or the adduction of the wing by the basalar sclerite brought about by the activity of *b1*. Due to the similar configuration of other muscle-sclerite complexes, we also hypothesise that the utilisation of sclerite-motion limitation is widespread across dipteran control muscles, with most if not all of the

muscles making use of this mechanism to some extent. Future work will be able to explore this idea in greater depth.

This study has exemplified the three main principles through which the control muscles influence wing movement: anatomy, activation timing, and mechanical advantage. These principles enable the fractional movement of small components to bring about large movements of larger components. Both micro- and nano-air vehicles are likely to encounter the same difficulties as insects in producing an adequate range of wing kinematics using only small actuators, and so the principles illustrated here will be relevant to the design of these machines.

There is still much to learn about the hugely complicated system that is the dipteran flight motor and the wing hinge, but this study illustrates techniques through which we can begin to tease apart these complexities. Future investigations will advance this technique with larger datasets and a wider range of muscles, and in doing so may identify further principles which can be applied to the bio-inspired engineering of vehicles and machines.

Appendices

Appendix E

Averaged Linear Model Results

Maximum Stroke Angle				
<i>Parameter</i>	<i>Estimate</i>	<i>Unconditional SE</i>	<i>Upper CI</i>	<i>Lower CI</i>
<i>Intercept</i>	13.566	3.357	20.111	7.021
<i>I1_{MaxLength}</i>	-1.791	1.030	0.218	-3.800
<i>I2_{MaxLength}</i>	-2.401	1.096	-0.264	-4.539
Minimum Stroke Angle				
<i>Parameter</i>	<i>Estimate</i>	<i>Unconditional SE</i>	<i>Upper CI</i>	<i>Lower CI</i>
<i>Intercept</i>	10.084	2.200	14.374	5.794
<i>b1_{Amp}</i>	4.572	2.002	8.476	0.668

Maximum Deviation Angle

<i>Parameter</i>	<i>Estimate</i>	<i>Unconditional SE</i>	<i>Upper CI</i>	<i>Lower CI</i>
<i>Intercept</i>	0.978	1.286	3.486	-1.530
<i>I1_{MaxLength}</i>	-1.249	0.418	-0.434	-2.063
<i>I1_{Offset}</i>	-1.226	0.441	-0.366	-2.086
<i>I1_{Amp}</i>	-1.226	1.317	1.342	-3.795

Minimum Deviation Angle

<i>Parameter</i>	<i>Estimate</i>	<i>Unconditional SE</i>	<i>Upper CI</i>	<i>Lower CI</i>
<i>Intercept</i>	2.430	4.041	10.309	-5.450
<i>I1_{Offset}</i>	-2.917	1.099	-0.775	-5.060
<i>I1_{MaxLength}</i>	-2.928	1.085	-0.812	-5.043
<i>b1_{Amp}</i>	-5.334	2.185	-1.074	-9.595
<i>b1_{Phase}</i>	-13.610	5.563	-2.762	-24.459

Mean Deviation Angle

<i>Parameter</i>	<i>Estimate</i>	<i>Unconditional SE</i>	<i>Upper CI</i>	<i>Lower CI</i>
<i>Intercept</i>	0.893	1.334	3.495	-1.709
<i>I1_{Offset}</i>	-1.697	0.475	-0.771	-2.622
<i>I1_{MaxLength}</i>	-1.678	0.459	-0.783	-2.573
<i>b1_{Phase}</i>	-5.131	2.295	-0.656	-9.606

Mean Supination Timing (Proportion of Wingbeat)				
<i>Parameter</i>	<i>Estimate</i>	<i>Unconditional SE</i>	<i>Upper CI</i>	<i>Lower CI</i>
<i>Intercept</i>	-0.010	0.014	0.017	-0.037
<i>I1_{MaxLength}</i>	-0.010	0.006	0.002	-0.020
<i>b1_{Offset}</i>	0.004	0.002	0.006	0.0005
<i>b1_{Phase}</i>	-0.077	0.025	-0.027	-0.126

Mean Stroke Plane				
<i>Parameter</i>	<i>Estimate</i>	<i>Unconditional SE</i>	<i>Upper CI</i>	<i>Lower CI</i>
<i>Intercept</i>	-2.727	1.527	0.2506	-5.705
<i>I1_{Offset}</i>	1.335	0.457	2.227	0.444
<i>I1_{MaxLength}</i>	1.210	0.448	2.085	0.336
<i>b1_{Amp}</i>	2.715	1.016	4.695	0.735
<i>b1_{Offset}</i>	-0.349	0.155	-0.048	-0.651
<i>b1_{Phase}</i>	4.635	2.699	9.897	-0.627

Maximum Downstroke Angle of Incidence				
<i>Parameter</i>	<i>Estimate</i>	<i>Unconditional SE</i>	<i>Upper CI</i>	<i>Lower CI</i>
<i>Intercept</i>	4.832	4.945	14.474	-4.810
<i>I1_{MaxLength}</i>	2.362	1.786	5.844	-1.121
<i>b1_{Phase}</i>	19.140	8.665	36.037	2.242

Minimum Downstroke Angle of Incidence				
<i>Parameter</i>	<i>Estimate</i>	<i>Unconditional SE</i>	<i>Upper CI</i>	<i>Lower CI</i>
<i>Intercept</i>	-0.614	1.306	1.932	-3.160
<i>b1_{Offset}</i>	-0.595	0.190	-0.225	-0.965
<i>b1_{MaxLength}</i>	-0.602	0.197	-0.218	-0.986
<i>b1_{Phase}</i>	5.195	2.614	10.292	0.099
<i>b1_{Amp}</i>	1.460	1.050	3.507	-0.588

Mean Downstroke Angle of Incidence

<i>Parameter</i>	<i>Estimate</i>	<i>Unconditional SE</i>	<i>Upper CI</i>	<i>Lower CI</i>
<i>Intercept</i>	-1.090	2.329	3.451	-5.632
<i>I1_{MaxLength}</i>	1.478	0.657	2.760	0.197
<i>I1_{Offset}</i>	1.370	0.712	2.760	-0.0183
<i>b1_{MaxLength}</i>	-0.570	0.221	-0.139	-1.001
<i>b1_{Offset}</i>	-0.612	0.207	-0.208	-1.017
<i>b1_{MaxLength}</i>	3.551	1.564	6.600	0.501
<i>b1_{Phase}</i>	10.411	3.746	17.715	3.107

Minimum Upstroke Angle of Incidence

<i>Parameter</i>	<i>Estimate</i>	<i>Unconditional SE</i>	<i>Upper CI</i>	<i>Lower CI</i>
<i>Intercept</i>	4.638	4.442	13.299	-4.024
<i>I1_{Offset}</i>	-3.689	1.431	-0.899	-6.478
<i>I2_{Amp}</i>	3.002	1.427	5.784	0.220
<i>b1_{MaxLength}</i>	-1.543	0.477	-0.613	-2.473
<i>b1_{Offset}</i>	-1.512	0.462	-0.611	-2.413
<i>b1_{Phase}</i>	19.117	7.368	33.485	4.749

Mean Stroke Amplitude

<i>Parameter</i>	<i>Estimate</i>	<i>Unconditional SE</i>	<i>Upper CI</i>	<i>Lower CI</i>
<i>Intercept</i>	-6.128	5.167	3.948	-16.204
<i>I1_{MaxLength}</i>	1.873	1.423	4.648	-0.902
<i>I2_{MaxLength}</i>	3.513	1.582	6.598	0.428
<i>b1_{Amp}</i>	7.578	3.552	14.503	0.652

Chapter 8

Conclusions and Outlook

The dipteran wing hinge is extremely complex, but this thesis has demonstrated how the use of cutting-edge techniques can enable us to visualise the system in ways that were previously impossible. This, in turn, has enabled us to determine the biomechanics of these remarkably complicated mechanisms that enable such precise wing kinematic control in dipteran insects.

Where there has previously been debate regarding the role and function of the interaction between the radial stop and pleural wing process that forms the gear change mechanism, our research here has made use of high speed videography, macrography and comparative anatomy to determine the wing kinematic changes that occur during this interaction, and how these changes are brought about. We have determined that the engagement of the *RS* and *PWP* influences the movement of the wing through acting as a pivot about which wing kinematics can be modulated. The anatomy of these components varies across species, however, and so does the degree of control this interaction affords as a result. The gear change mechanism is well developed in *Calliphora vicina*, in which three distinct gears (type of *RS-PWP* interaction) are possible, each associated with their own significantly different sets of wing kinematics. *Eristalis tenax*, however, shows a much smaller *PWP*, and only two gears are available. The kinematics associated with these gears are also less distinct compared to those of *C. vicina*, which we believe directly relates to the fact that the *PWP* is both absolutely and proportionally smaller, meaning it cannot be

used to impede the movement of the *RS* to the same extent as that of *C. vicina*.

Due to the gear change mechanism being less important in the control of wing kinematics in *E. tenax* than in *C. vicina*, it must therefore be the case that this species makes greater use of the control muscles that attach to the sclerites of the wing hinge to determine wing movement. Even in *C. vicina* though, these control muscles are perfectly capable of controlling the kinematics of the wing when the *PWP* has been experimentally removed, suggesting that the gear change mechanism is not an essential part of the flight motor. Instead, it may be a method of improving the efficiency of the system, providing a mechanism for coarse kinematic control while the control muscles perform fine adjustments (Chapters 3, 4 and 5).

Another element of the wing hinge, the Pterale C, has been hypothesised to be another element of the wing hinge that absorbs kinetic energy from the wing in order to control wing kinematics. However, our studies here have demonstrated not only that this component is poorly suited to absorb energy and restrict wing movement. Despite the *PtC* making extensive contact with the *RS* during the latter half of the downstroke, the timing of this contact does not correlate with any wing kinematics in such a way as to suggest it is mechanically restricting wing movement. Instead, we find that these correlations support a hypothesis in which the *PtC* is a proprioceptive structure, informing the insect of the position, and possibly the velocity of the wing as it approaches the end of the downstroke (Chapter 6). This information can then be used by the central nervous system of the insect, such as by inducing activity in the control muscles, with the first basalar muscle (*b1*) being a muscle of particular interest.

While the effects of control muscle activity, like that of *b1*, on wing kinematics have been determined by previous authors, the biomechanics through which these muscles change wing kinematics have never been fully understood. In this thesis, the anatomy and movement of the *I1*, *I2* and *b1* has been documented using time-resolved synchrotron X-ray computed microtomography, and we find that bilateral differences in strain patterns of these muscles correlate with bilateral differences

in wing kinematics. This information, combined with the knowledge gleaned from observing the motion of the muscles throughout the wingbeat, has allowed us to infer that all of these muscles act by restricting the movement of the wing hinge sclerites to which they attach. *I1* and *I2* restrict the rotation of *Ax1* at the end of the downstroke, presumably becoming more restrictive during contraction as the muscle becomes shorter (and likely stiffer), while *b1* controls the movement of the basalar sclerite by restricting its motion at the end of the upstroke. All of the control muscles studied here may therefore control wing kinematics by absorbing energy and inhibiting the motion of wing hinge components, and we believe that many of the other control muscles may act through similar mechanisms (Chapter 7).

The absorption of energy and the restriction of movement are recurring themes throughout the dipteran flight motor. The gear change mechanism acts to restrict wing movement by absorbing energy through *RS-PWP* interaction, and this ability facilitates its control over wing kinematics. In situations where either component is not adapted for this role of energy absorption and storage, the mechanism instead appears to be adapted to aid the control of wing rotation around the end of the downstroke, just as we observe when comparing the gear change mechanism between *C. vicina* and *E. tenax*. The control muscles of the flight motor also restrict the movement of the sclerites to which they attach, altering the way in which the work from the power muscles is translated into wing motion, and changing wing kinematics as a result. The dipteran flight motor is therefore a system in which large power muscles provide the energy to drive wing movement, and left unaltered this would produce bilaterally symmetrical, unrestricted wing movement. Through the involvement of the control muscles and gear change mechanism however, either or both wings have their movement restricted, determining the level of wing motion asymmetry.

That insects control wing kinematics through the use of motion inhibition rather than by having the control muscles contribute positive work likely relates to the benefits of eccentric muscle contraction and negative work over positive work. Though

I have not been able to show in this thesis that the control muscles utilise negative work, there is a large amount of literature outlining the increases in efficiency and efficacy of negative versus positive muscular work, and the advantages of the former (Fenn, 1924; Abbott et al., 1951, 1952; Bigland-Ritchie and Woods, 1976; Lindstedt et al., 2001; Elmer and LaStayo, 2014). Both of these are extremely important qualities for muscles that are required to operate for long periods of time during flight, and influence the movement of components when operating in a system dominated by other larger and more powerful muscles (i.e. the power muscles). These muscles are also able to implement other mechanisms, such as the gear change mechanism, which may increase both the efficacy and efficiency of their work. The dipteran flight motor is a highly evolved system, and it is therefore no surprise to find that the myriad elements within it have evolved to operate optimally.

In revealing the mechanisms of motion inhibition and energy absorption underlying the precise control of wing kinematics in dipteran insects we can begin to apply this knowledge to the design of micro- or nano-air vehicles, or other small-scale systems. In many terrestrial vehicles, suspension systems rely on the inhibition of motion, for example in cars to buffer displacements of wheels from the chassis, while brake pads can inhibit the motion of wheels by absorbing kinetic energy and dissipating it as heat. Similarly, motion inhibition mechanisms could be applied to the control of oscillatory movements. In the same way that *I1* and *I2* restrict wing movement by varying their length through contraction, variable-stiffness springs could be used to control ornithopter wings. Furthermore, if this energy is stored and returned to the system in the following cycle, it can be used to ensure that these systems operate at resonance with minimal input energy required to reverse the direction of wing motion, thereby increasing the efficiency of the system. More dedicated research into the engineering of these systems will determine the best ways in which this work can be applied to the design of bio-inspired machines.

This thesis represents significant steps forward in our understanding of the dipteran flight motor, revealing that the entire system may rely on the inhibition

of component motion to control wing kinematics. The work presented here opens up new avenues of research for bio-inspired designs, potentially identifying effective mechanisms for controlling the wings of flapping-wing N/MAVs. Future studies will have access to novel technologies, and in doing so will be able to elucidate even more about the biomechanics of these remarkable insects. This thesis, and the future work it will inform, will allow us to apply the properties and mechanisms found in the dipteran flight motor to bio-inspired vehicles which emulate one of the most complex and effective mechanical systems found in nature.

Chapter 9

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