Integrative and Comparative Biology

Integrative and Comparative Biology, volume 64, number 2, pp. 533–555
https://doi.org/10.1093/icb/icae106

Society for Integrative and Comparative Biology

SYMPOSIUM INTRODUCTION

Insect Flight: State of the Field and Future Directions

Lisa A. Treidel[*,1,2], Kevin D. Deem[†], Mary K. Salcedo[‡], Michael H. Dickinson[§], Heather S. Bruce[¶], Charles-A. Darveau[||], Bradley H. Dickerson[#], Olaf Ellers[**], Jordan R. Glass[††], Caleb M. Gordon[‡‡], Jon F. Harrison[§§], Tyson L. Hedrick[¶¶], Meredith G. Johnson[§§], Jacqueline E. Lebenzon[||||], James H. Marden[##], Kristjan Niitepõld[***], Sanjay P. Sane[†††], Simon Sponberg[‡‡‡], Stav Talal[§§], Caroline M. Williams[||||] and Ethan S. Wold[‡‡‡]

[*]School of Biological Sciences, University of Nebraska Lincoln, Lincoln, NE 68588, USA; [†]Department of Biology, University of Rochester, Rochester, NY 14627, USA; [‡]Department of Biological and Environmental Engineering, Cornell University, Ithaca, NY 14853, USA; [§]Department of Bioengineering, California Institute of Technology, Pasadena, CA 91125, USA; [¶]Marine Biological Laboratory, Woods Hole, MA 02543-1050, USA; [||]Department of Biology, University of Ottawa, Ottawa Ontario K1N 6N5, Canada; [#]Princeton Neuroscience Institute, Princeton University, Princeton, NJ 08544, USA; [**]Biology Department, Bowdoin College, Brunswick, ME 04011, USA; [††]Department of Zoology and Physiology, University of Wyoming, Laramie, WY 82070, USA; [‡‡]Department of Earth and Planetary Sciences, Yale University, New Haven, CT 06520-8109, USA; [§§]School of Life Sciences, Arizona State University, Tempe, AZ 85287-4501, USA; [¶¶]Department of Biology, University of North Carolina, Chapel Hill, NC 27599, USA; [||||]Department of Integrative Biology, University of California, Berkeley, CA 94720, USA; [##]Department of Biology, Pennsylvania State University, University Park, PA 16803, USA; [***]Heureka, the Finnish Science Centre, Vantaa 01300, Finland; [†††]National Center for Biological Sciences, Tata Institute of Fundamental Research, Bangalore 560065, India; [‡‡‡]School of Biological Sciences, Georgia Institute of Technology, Atlanta, GA 30332, USA

The first four authors contributed equally to this work.
From the symposium “Evolution, Physiology, and Biomechanics of Insect Flight” presented at the annual meeting of the Society for Integrative and Comparative Biology, January 2-6, 2024.

[1]E-mail: latreidel@wm.edu
[2]Present Address: Department of Biology, William and Mary, Williamsburg VA 23187, USA

Synopsis The evolution of flight in an early winged insect ancestral lineage is recognized as a key adaptation explaining the unparalleled success and diversification of insects. Subsequent transitions and modifications to flight machinery, including secondary reductions and losses, also play a central role in shaping the impacts of insects on broadscale geographic and ecological processes and patterns in the present and future. Given the importance of insect flight, there has been a centuries-long history of research and debate on the evolutionary origins and biological mechanisms of flight. Here, we revisit this history from an interdisciplinary perspective, discussing recent discoveries regarding the developmental origins, physiology, biomechanics, and neurobiology and sensory control of flight in a diverse set of insect models. We also identify major outstanding questions yet to be addressed and provide recommendations for overcoming current methodological challenges faced when studying insect flight, which will allow the field to continue to move forward in new and exciting directions. By integrating mechanistic work into ecological and evolutionary contexts, we hope that this synthesis promotes and stimulates new interdisciplinary research efforts necessary to close the many existing gaps about the causes and consequences of insect flight evolution.

Advance Access publication July 9, 2024

Introduction

*This manuscript was developed and written collaboratively as a component of the symposium "Evolution, Physiology, and Biomechanics of Insect Flight" held at the annual meeting of the Society of Integrative and Comparative Biology in Seattle Washington (USA) on Jan 2–6,2024.

Approximately 400 million years ago, an evolutionary innovation arose that fundamentally altered the history of life. A simple set of protowings evolved in a basal member of the Pterygota (winged insect) lineage (Misof et al. 2014). The exact origin, morphology, and use of protowings are still a matter of debate (Hamilton 1971; Kukalova-Peck 1978; Rasnitsyn 1981; Marden et al. 2000; Dudley et al. 2007; Prokop et al. 2017; Tihelka et al. 2021; Ross 2022). Nonetheless, wing evolution undoubtedly set the stage for subsequent adaptations that further augmented their function, eventually giving rise to active-powered flight within insects. Active flight provides adaptive benefits of dispersal with a low cost of transport (Tucker 1975), including enhanced access to resources, mating opportunities, and new habitats, making the evolution of flight arguably the critical adaptation central to the astonishing success and diversification of insects (Engel et al. 2013; Nicholson et al. 2014). Unsurprisingly, then, the study of insect flight has attracted the interest of biologists for over two centuries. However, flight is a complex trait that requires coordinated innovations from many aspects of organismal biology, including morphology, biomechanics, neurobiology, skeletal muscle form and function, metabolism, and development to evolve. This complexity, along with large gaps in the fossil record, has posed a major challenge, leaving many open questions about how and why insect flight evolved.

Recent cross-disciplinary work integrating comparative, developmental, physiological, and biomechanical perspectives and novel experimental approaches has yielded new insights on the mechanisms shaping evolutionary patterns of insect flight. First, advances in evolutionary genetics and developmental biology have allowed us to test long-standing and conflicting hypotheses on the homologous origins of insect wings (e.g., Bruce and Patel 2020; Clark-Hachtel and Tomoyasu 2020; Ohde et al. 2022). Second, we are beginning to explore the roles of developmental, physiological, and behavioral plasticity in driving the evolutionary diversification of flight among a broad range of taxa (e.g., Niitepõld 2019; Du et al. 2022; Glass et al. 2024). This work promises to help explain the influence of environmental variation on determining variation in flight performance among individuals and species, providing a currency upon which evolution can act. Third, work investigating how the sensory and neuromuscular systems are functionally integrated to control the mechanics of the thorax, wing hinge, and wing internal structures, is leading to new discoveries explaining how diverse and robust flight strategies and maneuvers arise (e.g., Lindsay et al. 2017; Tsai et al. 2020; Melis et al. 2024).

In complement to the papers that follow in this Symposium issue, the goal of this manuscript is to highlight recent developments and exciting directions for the field of insect flight that benefit from an interdisciplinary approach. We do not provide a comprehensive review of the literature. Instead, we focus on linking together the fields, research interests, and expertise of the authors by highlighting and discussing unresolved debates and outstanding cross-cutting questions on the evolution, physiology, biomechanics, and neurobiology of insect flight. In addition, many questions remain unanswered due to challenges and technical limitations associated with studying complex flight behaviors, particularly in nature and insects, many of which are non-model organisms. Thus, we also strive to identify, provide recommendations, and highlight examples of work moving the field forward in new directions by using novel and integrative approaches to overcome these challenges.

Evolutionary origins and dynamics of the flight apparatus

The debate regarding the evolutionary origin of the insect wing has an embroiled history spanning more than 200 years (Latreille 1819; Crampton 1916). Historically, inferences about evolutionary events were made solely via morphological comparisons of fossilized and living members of lineages that arose before or after wings originated (Crampton 1916). The earliest definitive fossils of winged insects date to the early Carboniferous (∼325 Myr ago) (Prokop et al. 2005; Prokop et al. 2017; Prokop et al. 2022), but given that these species already had wings, the morphologies of the earliest insect wings and wing precursor structures remain unclear. Molecular clock estimates suggest an Early Devonian (419–393 Myr ago) origin of insect flight (Misof et al. 2014), making the identification of Devonian and Lower Carboniferous fossils a priority for paleoentomology (Jarzembowski 2021).

Two prominent hypotheses on the anatomical origin of wings dominated much of the paleontological literature, positing that wings either evolved from extensions of the lateral terga (paranotal theory; Crampton 1916; Hamilton 1971) or from side lobes (exites, such as plates and gills) on the base of the ancestral leg that now forms the lateral body wall (pleura) of insects (exite theory; Kukalova-Peck 1978). Because both the paranotal lobe and the leg exite theories had much evidence in their favor, a third theory was proposed: the dual origin hypothesis (Rasnitsyn 1981) argued that wings evolved with contributions from both lateral tergal and pleural tissues.

Advances in molecular techniques in evolutionary and developmental biology aimed at identifying wing serial homologs in insects and homologs in crustaceans have led to new clarity on these alternative wing origin theories (Averof and Cohen 1997; Niwa et al. 2010; Clark Hachtel et al. 2013; Ohde et al. 2013; Medved et al. 2015; Lozano-Fernandez et al. 2019; Ohde et al. 2022). Patterns of gene expression and morphological responses to gene manipulations, support the view that the pleura and lateral terga of insects correspond to the base of the leg in crustaceans (Bruce and Patel 2020; Clark-Hachtel and Tomoyasu 2020) (Fig. 1). This suggests that the ancestral crustacean leg base broadened to become the insect lateral body wall, and that this broadening carried the precoxal exite dorsally, where it formed first the paranotal lobe and then later the wing (Kobayashi et al. 2022) (Fig. 2). In this model, both the exite and paranotal theories are correct—not because pleura and terga merged as in the dual origin theory—but rather because the exite and paranotal theories pertain to different phylogenetic timepoints, as follows: the insect wing ultimately evolved from the precoxal exite (tergal plate) on the ancestral leg base—consistent with the leg exite theory; then the leg base became the lateral body wall, and the precoxal exite evolved into the paranotal lobe; and finally, the paranotal lobe evolved into the wing—consistent with the paranotal theory. This paranotal theory was subsequently broadened into a "precoxal theory" by Kobayashi et al. (2022) to reflect that the lateral terga on which wings and paranotal lobes stand is itself derived from the ancestral precoxa.

The recent discovery that wings evolved from body wall, which originated as the proximal precoxa leg segment, provides a number of new insights that presage further scientific inquiry. First, we more fully understand the anatomical origin and roles of several key functional genes underlying the development of wings. This information puts to rest any notion that wings evolved from tissue that had no prior evolutionary history of having plates, muscles, articulations, nerves, and movement. Thus, despite this increased clarity on the anatomical origins of wings themselves, the origin of other essential components of the flight apparatus remains enigmatic. Insect flight requires an integrated biomechanical system, including flight musculature, articulation at the wing hinge between resilin-laden thorax and wings (and sometimes halteres), and a functioning wing with its hemolymph, nerves, and tracheae (Ellington 1984; Dudley 2000). Much work remains to be done to elucidate how a locomotory leg base with

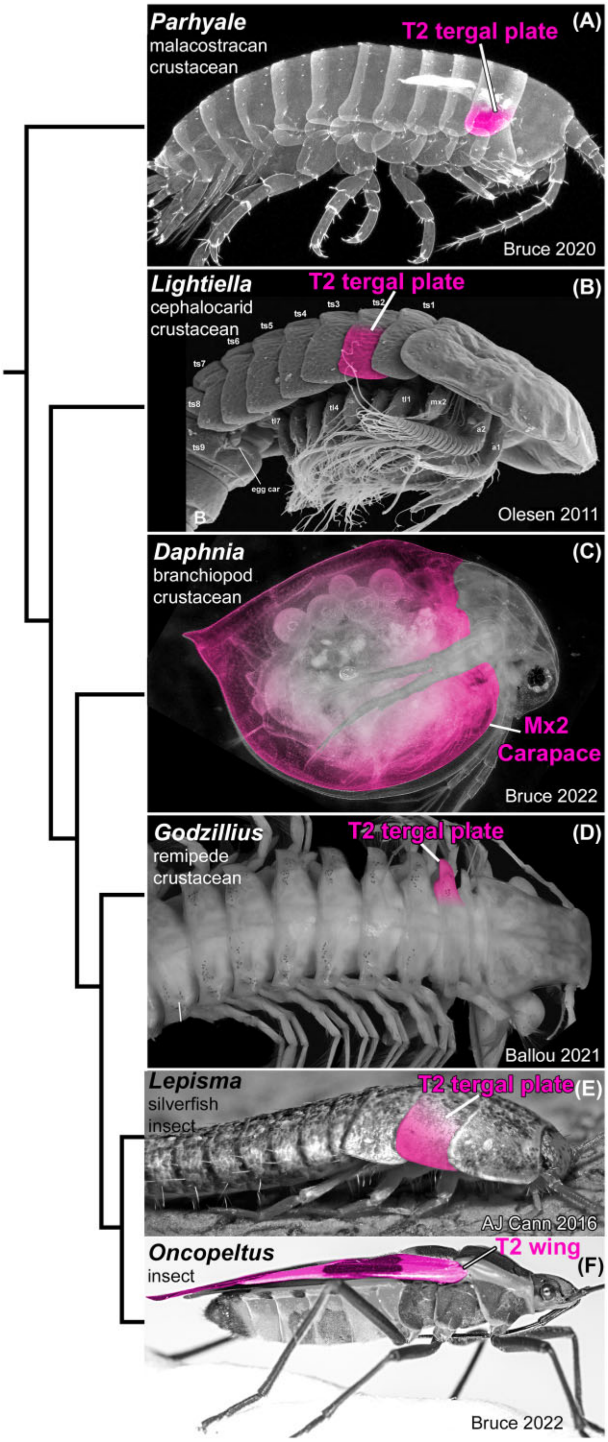


Fig. 1 Proposed exites on the precoxa (leg segment 8) in representatives of pancrustacean lineages near the base of the hexapod phylogenetic tree. These precoxal exites (labeled as T2 tergal plate (A,B,D,E) or Mx2 carapace (C)) appear to be non-respiratory tergal plates (A,B,D,E) and similar to rigid ectodermal plates, like the Daphnia carapace (C). Given that the insect wing is also a non-respiratory exite on the precoxa (F), this phylogenetic sequence suggests that insect wings evolved from a tergal plate rather than a respiratory gill. Phylogeny based on Lozano-Fernandez et al. (2019). Oncopeltus image credit: Aaron Pomerantz.

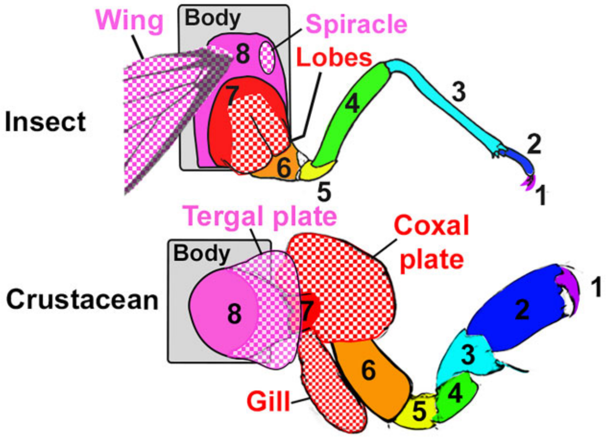
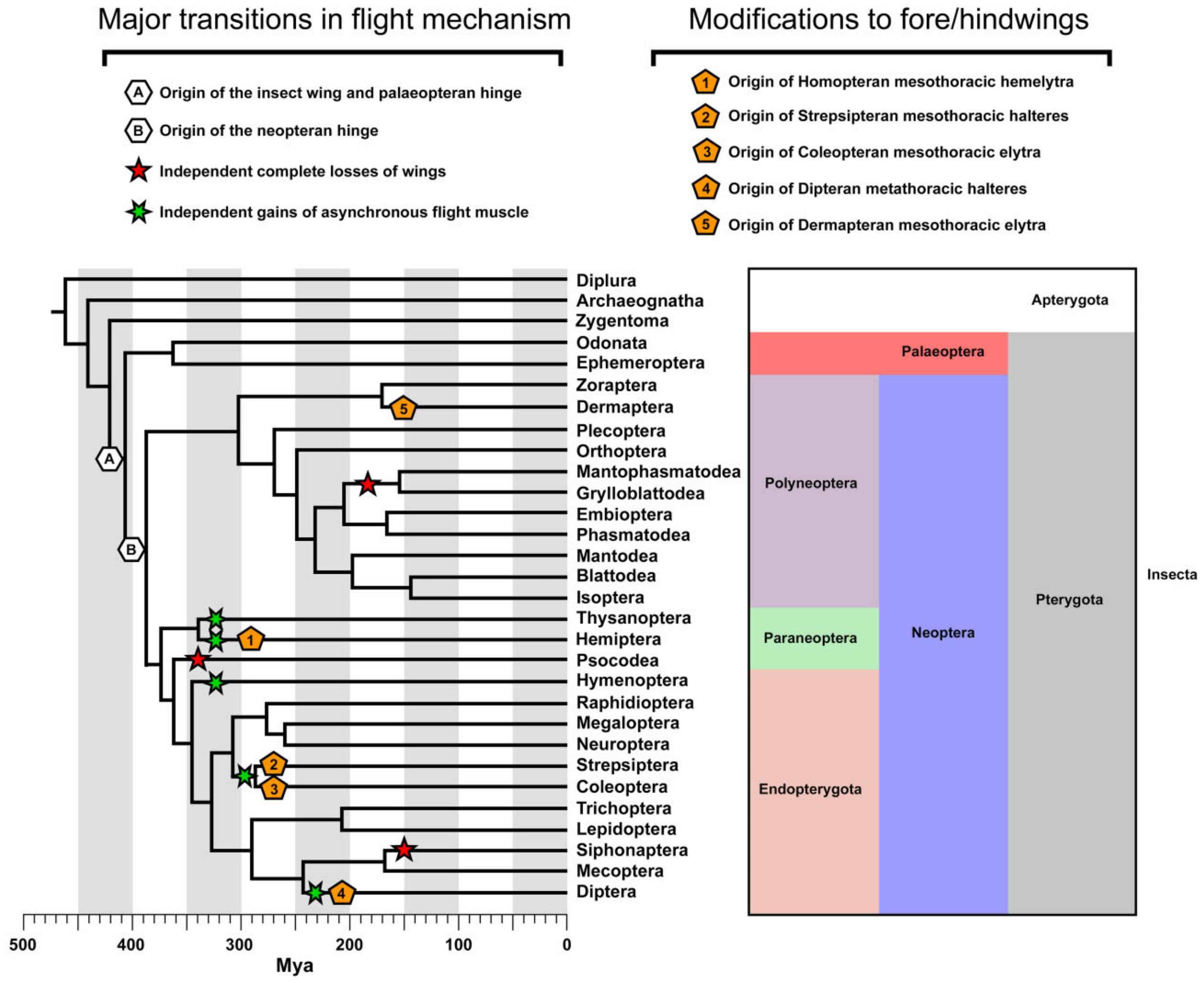


Fig. 2 Diagram of homologies of crustacean and insect leg segments based on the expression and function of leg gap genes and wing/exite genes in Bruce and Patel (2020) and Bruce and Patel (2022). Colors indicate proposed homologies, checker pattern indicates exites. Insect leg drawing modified from Snodgrass (1935). Panel modified from Bruce and Patel (2020).

its exite was further modified into a flight-capable system. For example, are flight muscles derived from only leg muscles of the precoxa, or are there additional muscle contributions from the pleura or the dorsal-medial, non-leg-derived terga? How did the wing hinge—a region of flexible, convoluted ectoderm that allows the wing to flap—originate from an initially smooth cuticle? How did the stiffening veins of the wings evolve, and can we find their counterpart in crustacean exites? Furthermore, much of the historical and current debate surrounding insect wing origins focuses on the ectoderm, but the evolution of powered flight must have involved complementary evolution in the musculature and neural circuitry. How were the ancestral locomotory leg base muscles and neural circuitry modified for flight? Given that the wing appears to be derived from a leg exite of a crustacean ancestor, future experiments should compare the muscles and motor neurons involved in insect flight to those of the crustacean leg base.

The initial function and selective pressures leading to the evolution of wings and flight also remain an area of active research, with the two main alternatives being "trees-down" (terrestrial origins of wings) or "water-up" (aquatic origin of wings). The "trees-down" model posits that winglets were used to control aerial descent when falling or jumping from plants (Dudley and Yanoviak 2011). This model is supported by experiments showing that abdominal cerci or legs can modestly deflect the path of falling bristletails and ants to accomplish directed descent from plants or cliffs (Yanoviak et al. 2005; Yanoviak et al. 2009). Additionally, recent morphological and phylogenomic studies have advocated for a terrestrial ancestor for the Pterygotes (winged insects) (Wipfler et al. 2019), consistent with the "trees-down" model. However, this phylogenomic analysis excluded the crustacean-like ancestral state and many uncertainties remain, such that the alternative of an aquatic or semi-aquatic ancestor of flying insects predicted by the "water-up" model cannot be ruled out (Averof and Cohen 1997; Simon et al. 2018; Tihelka et al. 2021; Prokop et al. 2023). The "water-up" model posits the evolution of wings was selected for in a semiaquatic insect ancestor that initially used winglets to aid respiration during aquatic larval stages and were modified to support aerodynamic locomotion. This model is supported by evidence from live mayflies and stoneflies showing winglets and wings are used for steering and propulsion on water surfaces without the need for weight-supported flight (surface-skimming) (Marden and Kramer 1994; Marden and Kramer 1995; Marden et al. 2000; Marden and Thomas 2003), and there is a close match of surface-skimming with behavioral evidence from the earliest trace fossil of a winged insect (Marden 2013a). The absence of gills on the prexcoa leg segment that insect wings evolved from in their most closely related crustacean clades (Fig. 1), encourages a revision of the literal "wings-from-gills" version of the "water up" model. However, this evidence does not preclude a scenario where protowings are moveable and have a ventilatory function, as in the "water-up" model. Future work integrating phylogenomic analyses, new fossil evidence, development, physiology, behavior, and ecology will be necessary to differentiate these alternative adaptive origin of flight hypotheses.

Following the origin of insect flight, wings and associated flight machinery have undergone continued diversifications, including several major macroevolutionary transitions (Fig. 3). Indeed, the flight mechanism is as hyper-diverse as the insects themselves and likely evolved in close association with the diverse ecological functions of flight (Hörnschemeyer and Willkommen 2007; Aiello et al. 2021). Major modifications include the evolution of wing hinge function, allowing neopteran lineages to have wings that fold backwards along their body (Martynov 1925), as well as reduced and modified wings such as halteres in Diptera (Deora et al. 2021) and elytra in Coleoptera (Linz et al. 2016; Sugimoto et al. 2018; Goczał and Beutel 2023). One important macroevolutionary transition that we have just begun to resolve is the evolution of an asynchronous neural drive of flight muscles. Ancestral insects generated wing beats through the synchronous activation of wing muscles with each wing stroke. However, many clades such as Diptera, Coleoptera, and Hymenoptera have evolved the ability to generate wingbeat frequencies above the speed



1. Misof et al. (2014) "Phylogenomics resolves the timing and pattern of insect evolution. Science. **346**(6210): 763-767
2. Martynov, A. V. (1923). "О двух основных типах крыльев насекомых и их значении для общей классификаци насекомых" [On the two main types of insect wings and their significance for the general classification of insects]. Proceedings of the I All-Russian Congress of Zoologists, Anatomists and Histologists in Petrograd on 15-21 December 1922: 88–89.
3. Martynov, A. V. (1924). "О двух типах крыльев насекомых и их эволюции" [There are two types of drug addicts and evolutionists]. Russian Zoological Journal. 4 (1, 2): 155–185
4. Dudley (2000) The Biomechanics of Insect Flight: Form, Function, and Evolution. Princeton University Press, Princeton, NJ. ISBN 0-691-09491-8

Fig. 3 Phylogeny of insect orders with major macroevolutionary transitions and modifications in the wings and flight machinery, responsible for the diversification of flight ability and strategies among insects.

limits for typical neuromuscular systems by using asynchronous stretch-activated muscle physiology that decouples their wingbeat frequency from their underlying neural drive (Josephson et al. 2000). In these insects, neural activity serves mostly to potentiate muscle force on multiple-wingbeat timescales, and the stretch response of antagonistic flight muscles produces each wing stroke. A recent ancestral state reconstruction of muscle type across an insect-wide phylogeny revealed a single origin of asynchrony followed by many reversions from asynchronous flight back to synchronous flight, leaving entire clades such as Lepidoptera secondarily synchronous (Gau et al. 2023). More importantly, the two modes of muscle actuation can be mixed in a single species, and transitions from synchronous to asynchronous wingbeats map to simple transitions in an underlying biophysical model (Gau et al. 2023). This suggests that transitions may be easier and more frequent than the specialized asynchronous mechanisms suggest at first glance. At a broad scale, the evolution of flight muscle asynchrony enabled high contraction rates and enhanced energetic efficiency in some clades (Cao and Jin 2020; Mesquita et al. 2021; Hickey et al. 2022).

Additionally, most insect orders contain both flight polymorphic and completely flightless species, which have secondarily lost some or all of their wings and flight machinery (Harrison 1980; Roff 1986). The question about why flight is so frequently and repeatedly lost has garnered much attention and is of particular significance because of potential evolutionary consequences of flight loss for speciation (Roff 1990; Waters et al. 2020). The first evolutionary hypotheses for flightlessness even date back to Darwin, who postulated that high winds drive displacement of insects from oceanic islands leading to the loss of flight (Darwin 1859). Flight-capable insects incur high energetic costs when developing and maintaining flight machinery and actively flying, which result in trade-offs between flight and other life history traits such as reproduction and somatic maintenance (Rankin and Burchsted 1992). Thus, selection for flightlessness is favored in a variety of ecological contexts where either the costs of flight become too high, such as in fragmented habitats (e.g., in alpine Stoneflies (Foster et al. 2021), or benefits of flight are reduced, such as in stable habitats (see Leihy and Chown (2020) for a table of supported ecological hypotheses).

Apparent evolutionary reversals of flight loss are driving us to further develop evolutionary models for complex trait evolution. Several phylogenetic reconstruction analyses suggest that wings have putatively independently re-emerged in multiple lineages of water striders, stick insects, and male aphids following their complete loss (Andersen 1993; Whiting et al. 2003; Bank and Bradler 2022; Forni et al. 2022; Saleh Ziabari et al. 2023). However, these patterns challenge Dollo's law of irreversibility, wherein a complex trait, once lost, will never return to the same state (Dollo 1893), and results of ancestral trait reconstruction analyses are easily biased by poor taxonomic resolution and the assumption of parsimony, making these conclusions controversial (Trueman et al. 2004). Nonetheless, the complexity and pleiotropy of the gene regulatory networks underlying wing development may explain these apparent evolutionary reversals of wing loss. Wing development pathways are composed of many transcription factors and signal transduction pathways (Linz et al. 2023), and any combination of nodes in the network may be environmentally sensitive and serve as targets for selection on wing diversification or loss. Thus, flight polymorphisms may evolve as environmentally controlled polyphenisms, single-loci Mendelian traits, or as polygenic traits under the control of many genes and the environment (Roff 1986; Roff and Fairbairn 1991). Furthermore, many members of the wing gene regulatory network are highly pleiotropic. For instance, the critical wing gene *apterous* is also involved in development of the terga (Cohen et al. 1992), legs (Pueyo et al. 2000), central nervous system (Aranha et al. 2017), flight muscle (Bernard et al. 2003), and juvenile hormone synthesis (Altaratz et al. 1991). In wing polymorphic crickets, short or wingless morphs are produced by the manipulation of hormones involved in metamorphosis upstream of the wing gene regulatory network. Specifically, modulation of juvenile hormone and insulin-like signaling pathways is responsible for induction of short-wing morphs in crickets, brown planthoppers, and soapberry bugs, while the hormone ecdysone is involved in the pea aphid wing polyphenism (Xu et al. 2015; Zera 2016; Vellichirammal et al. 2017; Fawcett et al. 2018; Lin and Lavine 2018). Thus, it is likely that most modifications to insect wings come from changes in gene regulation during development, not the loss of these genes.

Gene duplication and divergence are also likely involved in many of the modifications to, losses of, or reemergences of the flight apparatus. Following gene duplication, the redundancy of the resultant paralogs gives evolution *carte blanch* to experiment with novel expression domains and protein functions. For example, early ancestral gene duplication events led to multiple copies and the diversification of the insulin receptor family, which have been differentially co-opted to control wing morph determination in planthoppers, linden bugs, and soapberry beetles (Xu and Zhang 2017; Fawcett et al. 2018; Smýkal et al. 2020). In pea aphids, a duplicated *follistatin* gene present on the X-chromosome is responsible for genetically determining wingless males (Li et al. 2020). This is one of three *follistatin* copies resulting from two duplication events within the last 40 million years. Unfortunately, due to their recent divergence, the three paralogs have highly similar coding sequences, causing issues with genome assembly and the design of qPCR primers and *in situ* hybridization probes. These technical problems are common for recently duplicated genes, making them difficult to identify and functionally analyze. Thus, young paralogs often go overlooked despite their evolutionary importance, making them an understudied and exciting research frontier for the evolution and loss of the flight machinery (Deem and Brisson 2024).

Diversity in physiological and biomechanical determinants of flight performance

Flight performance is powered by the integration of multiple physiological systems, organs, and tissues, including the wings and wing hinge, flight and steering muscles, metabolism, neuroendocrine control with sensory feedback, oxygen and carbon dioxide transport by the tracheal system, and fuel delivery by the cardiovascular system. Thus, research on the physiology and biomechanical mechanisms determining insect flight performance remains central to elucidating the functional constraints and trade-offs shaping widespread variation in flight performance among individuals, populations, and species. Flight performance is an umbrella term used to describe different components of flight, including take-off ability, lift production, speed, agility and maneuverability, stability, and endurance. The most relevant aspects of performance to fitness vary widely according to ecological niche: endurance and speed are highly relevant to highly mobile and strong-flying migratory insects; lift is critical for insects that carry loads such as nectar or pollen; maneuverability is key to avoiding predation; and stability is critical to hovering insects such as hawkmoths.

Measuring different aspects of flight performance among insects remains a major challenge. Current approaches fall into two categories: use of tethered individuals or free-flying individuals. Tethered flight experiments performed using flight mills and simulators measure variation in flight duration of highly mobile and strong-flying migratory insects, and species with a strong tarsal reflex promoting flight (Mouritsen and Frost 2002; Nesbit et al. 2009; Minter et al. 2018; Naranjo 2019). However, attaching insects to tethers requires stressful manipulation with potential negative consequences for behavior and motivation, does not require the animal to generate sufficient lift to fly, and often does not elicit maximal capacities for flight (Heinrich 1971; Rothe and Nachtigal 1989; Wolf et al. 1989; Dudley 1995; Dickinson et al. 1999; Glass and Harrison 2022). Flight chambers (e.g., wind tunnels, virtual reality landscapes, rapidly shaken containers) and laboratory free flight experiments are frequently used in biomechanical studies of flight behavior (Henningsson and Bomphrey 2011) and are also suited for assessment of flight endurance and metabolic rates (Harrison and Roberts 2000; Suarez 2000; Darveau et al. 2005b; Niitepõld 2010). Broad conclusions about flight performance should be avoided if tests measure a narrow aspect of flight, and there is a need to better establish connections between multiple aspects of flight performance in the lab and natural field settings (Ducatez et al. 2012; Cibotti et al. 2024).

Body size and scaling

The body size range of modern insects can span three orders of magnitude, and some extinct flying insects reached even greater sizes, with tremendous consequences on their flight function and ability (Lapina et al. 2021; Ellers et al. 2024). Required flight forces scale as L^4 (where L is the length scale), but mass scales only as L^3 (Deora et al. 2017). This means that to achieve adequate flight forces, smaller insects must either enhance their wing stroke amplitudes or their wing beat frequencies. Increasing wing length relative to the body size can increase wing stroke amplitude but this is often limited by the fact that the wings eventually collide into each other (Lighthill 1973; WeisFogh 1973). Thus, a widely observed pattern is that smaller insect taxa have higher wingbeat frequencies and consequently higher mass-specific flight metabolic rates (Greenewalt 1962; Casey 1981; Byrne et al. 1988; Dudley 2000; Darveau et al. 2005b; Tercel et al. 2018). However, recent work showed that stingless bee species do not shift wingbeat frequency over an order of magnitude variation in body size, instead showing larger wings and reduced mass-specific flight cost in smaller species (Duell et al. 2022). Also, at approximately 60 mg body size, the scaling of flight metabolic rates changes from hypermetric (i.e., slope > 1 or above isometry) for smaller (< 60 mg) insects to hypometric (i.e., slope < 1 or below isometry) for larger (>60 mg) insects (Duell et al. 2022). The causes of scaling relationships between body size and flight metabolic rate generally remain poorly understood (Bejan and Marden 2006). However, these data suggest that changes in Reynold's numbers with size are a critical factor determining mechanical and energetic costs of flight and warrant further consideration.

There is also a great need to investigate how morphological and physiological systems evolve across insects of different sizes to produce and sustain diverse flight behaviors in a variety of ecological niches. Adaptations in musculoskeletal systems and wing morphology likely evolve in correlation with body size and life history to ensure efficient flight and aerodynamic performance. For example, adaptive correlated evolution of body size, wing morphology, and life history accompanied the divergence of the sister hawkmoth (Sphingidae) and silkmoth (Saturniidae) clades (Aiello et al. 2021). Hawk moths evolved smaller wings capable of supporting high wing beat frequencies and maneuverability at a reduced power requirement, beneficial for feeding efficiently by hovering flight. In contrast, silk moths evolved larger wings and longer thoraxes that reduce wing loading and enhance their agility, beneficial for evading predators by erratic flight maneuvers (Aiello et al. 2021). The intertwined evolutionary histories of insect body size, wing morphology, and flight maneuvers also extend back hundreds of millions of years. For example, changes in wing morphology and wingbeat frequency may be required to explain how the largest flying insects ever (the Carboniferous and Permian griffenflies) generated sufficient lift for steady-state flapping flight (Ellers et al. 2024).

Well-established scaling relationships on how body size and wing shape affect flight aerodynamics and performance across diverse flying animals (Pennycuick 2008; Deakin 2010; Duell et al. 2022) have allowed for testing of scaling principles connecting insect form and function. To further examine how these multiple traits interact and evolve, usage and development of mathematical models and explorations in insect morphospaces that incorporate genetic frameworks are needed. In addition, combining data from molecular phylogenetics, wing morphology, wing beat frequency, and metabolic rate can help resolve finer scales of variation at the single clade and individual levels (Casey et al. 1985; Darveau et al. 2005b; Billardon and Darveau 2019; Duell et al. 2022).

Energetics, kinematics, and resonance

Variation in flight muscle structure and function is one of the strongest contributors to variation in flight performance (Marden 2000). Flying insects achieve the highest mass-specific metabolic rates in the animal kingdom, and insect flight muscle has extremely high mitochondrial and tracheolar content (Sacktor 1961; Beenakkers et al. 1984; Suarez 2000; Iwamoto 2011). However, we still lack an understanding of the factors that create variation in flight metabolic rates not accounted for by body mass and the implications of metabolic variation for flight performance (Harrison and Roberts 2000; Suarez 2000). Variation in flight performance across populations and species has been associated with flight muscle mitochondrial content in butterflies (Rauhamäki et al. 2014; Niitepõld et al. 2022) and hymenopterans (Hedges et al. 2019). However, conditions used (e.g., substrates provided) when measuring muscle oxidative capacity lead to varying estimates (Teulier et al. 2016; Menail et al. 2022; Wilmsen and Dzialowski 2023) that are not easy to reconcile with whole animal flight performance. Similarly, phylogenetic comparison using multiple species of bees and several indicators of muscle aerobic capacity failed to show straightforward associations between flight metabolic rate and tissue aerobic capacity. Instead, glycolytic flux capacity and membrane composition showed correlated evolution with species flight metabolic rate (Darveau et al. 2005a; Rodríguez et al. 2015). Across species, hovering flight metabolic rate and wingbeat frequency have consequences for flight muscle contraction efficiency (i.e., the ratio of ATP turnover to mechanical power output of contraction; Askew et al. 2010), further raising questions regarding energetic and performance consequences of contraction frequency regime. Together, these findings highlight the importance of studies connecting the muscle structural and functional elements with the diversity and evolution of flight energetics and performance across insect species.

Another major contributor to variation in flight metabolism and performance lies in allelic variation in genes encoding proteins involved in core metabolic pathways (Marden 2013b). Across populations and species of butterflies, variation in alleles of metabolic enzymes and oxygen-signaling systems affects tracheole development in flight muscles, explaining antagonistic pleiotropic effects genotype on flight performance and other life history traits (Watt et al. 2003; Wheat et al. 2005; Marden et al. 2013; Mattila 2015; Pekny et al. 2018; Marden et al. 2021). This variation has further been linked to organismal dispersal rates, metapopulation size, and stability in the Glanville fritillary butterfly, demonstrating a critical role of flight evolution in the eco-evolutionary dynamics of insects (Hanski et al. 2017). Similar to butterflies, honeybees and Drosophilids also show associations between metabolic alleles and flight metabolic rates (Harrison et al. 1996; Montooth et al. 2003), suggesting widespread genetic variation in metabolism related to flight performance, which could be targets of selection.

The energetic cost and efficiency of flight are also strongly influenced by the structure and biomechanics of the flight apparatus. The discovery of elasticity in the insect thorax in the mid-20th century (Weis-Fogh 1960) led to the conceptual model of flapping insects as mechanical resonators (Sotavalta 1952; Greenewalt 1960; Weis-Fogh 1973)—spring-mass systems with aerodynamic dissipation forced by the flight musculature and resulting in a resonance curve (amplitude vs. frequency). Resonance implies a limited range of energetically favorable frequencies where flapping is most aerodynamically efficient. As a consequence, modulating frequency may incur large energetic costs. This apparent tradeoff between efficiency and agility presents a problem for insects that must balance the two to ensure survival, but until recently, few studies have directly measured resonant properties of insects. Recent work in the hover-feeding hawkmoth *Manduca sexta* shows that this insect flaps significantly above its resonance frequency and is able to modulate its wingbeat frequency by up to 30% from wing stroke to wing stroke (Gau et al. 2021; Gau et al. 2023). Independent measurements of resonance in the thorax of honeybees suggest that they may be operating close to their resonant peak (Jankauski 2020). As such, it seems that different groups of insects may negotiate resonant performance tradeoffs differently. In some species, the series elasticity in the wing hinge may also be significant, especially for insects on the scale of *Drosophila* or smaller (Pons and Beatus 2022; Casey et al. 2023). Series elasticity can dramatically widen the resonance curve, enabling efficient flapping over a range of frequencies. Few studies of insect resonance have explicitly incorporated asynchronous muscle. However, a pair of recent studies show that resonance in tiny, asynchronous insects like *Drosophila* appears to be dominated by the characteristic high stiffness of asynchronous muscle as opposed to exoskeletal elasticity, enabling nonlinear resonant phenomena (Pons 2023; Pons et al. 2023).

At the organismal level, the distinct energetic, kinematic, and aerodynamic requirements of alternative flight behaviors and strategies give rise to distinct selective pressures with direct evolutionary consequences for the diversification of insect flight machinery and physiology. For instance, long-distance migratory flight requires adaptations to permit extended flight activity. The transoceanic migration of the dragonfly species, *Pantala flavescens*, is associated with an energetics-based time constraint, suggesting that migrating insects experience strong energetic limitations on flight times (Ranjan et al. 2023). How can longer-duration flights be achieved? An emerging hypothesis is that long-distance migrators evolve behavioral and physiological adaptations that increase flight efficiency and reduce energetic costs of prolonged flight. For example, monarch butterflies often engage in energetically inexpensive gliding behaviors during migratory flights (Gibo and Pallett 1979), and lower flight metabolic rates exhibited by monarchs from migratory compared to non-migratory populations are potentially explained by adaptive selection on genes regulating flight muscle development and efficiency (Zhan et al. 2014). Similarly, gregarious locusts, which are long-distance migrators, fly for longer and generate less reactive oxygen species (ROS) than their solitary counterparts by flying slower, reducing their flight metabolism (Du et al. 2022). At the cellular level, gregarious locusts have lower catabolic enzyme activities than solitary locusts; supporting the hypothesis that long-duration flight requires lower oxidative metabolic rates to reduce oxidative damage to the flight muscle (Du et al. 2022). While migrating locusts depend heavily on lipid metabolism to fuel prolonged flight (Weis-Fogh and Uvarov 1952), tethered locusts stop flight despite having a strong supply of body lipids (Du et al. 2022; Talal et al. 2023). The cause of this apparent exhaustion is not known, but may involve declining motivation, limits on the capacity to release diglycerides from the fat body, running out of more easily metabolizable unsaturated fatty acids, or oxidative damage. Future research testing these alternative hypotheses will provide new insight into the physiological constraints and mechanisms determining an important component of flight performance, endurance, and maximum flight times.

The complexity of the wing hinge

At the wing and thorax junction lies a complex, intricate wing hinge that varies in size and composition across insects (Boettiger and Furshpan 1952; Pringle 1957; Miyan et al. 1985; Ennos 1987; Wisser 1988). Extraordinary but experimentally frustrating, the wing hinge is composed of one continuous sheet, consisting of tiny hard sclerites embedded within a more flexible exoskeleton. Since wings themselves contain no muscles, regulation of kinematics must be accomplished by actions of small control muscles that pull on the sclerites at the base of the wing. The mechanical operation of the hinge remains enigmatic because basal sclerites are difficult to see and move so rapidly that their motion during flight has not been accurately captured despite extensive efforts using stroboscopic photography (Nalbach 1989), high-speed videography (Walker et al. 2012), or X-ray tomography (Walker et al. 2014).

The exact mechanisms by which the hinge transforms power and muscle strain into wing motion remains unclear. However, recent insight comes from studies on the direct steering muscles regulating hinge mechanics via their action on the wing sclerites. These control muscles are roughly stratified into two functional groups: small tonically active muscles that continuously trim wing motion for stable flight, and large, phasically active muscles that are recruited during rapid maneuvers (Lindsay et al. 2017). Muscles inserted on the wing sclerites are capable of eliciting different changes in the pattern of wing motion, which collectively forms an actuator system capable of generating a diverse array of flight maneuvers. Remarkably, in flies (Diptera), this system achieves dynamic aerial agility with a small number of control muscles, each innervated by just a single excitatory motor neuron (Cheong et al. 2023b). These features are expected to be either conserved or independently evolved in the diverse range of insects that have miniaturized.

Furthermore, recent experiments demonstrate a key coupling controlling wing hinge function of Dipterans. In flies, the motion of wings and halteres is mechanically coupled by the indirect flight muscles and the sub-epidermal ridge to ensure that the wings and halteres beat at identical frequencies, functioning as a dual-coupled oscillator system driven by thoracic linkage elements (Deora et al. 2017; Deora et al. 2021). Coupling ensures a strict phase relationship between the two structures that is likely essential for proper temporal integration of afferent mechanoreceptors on the wing and haltere, and proper phase tuning of flight motor neurons. Moreover, this work solves an important riddle as to how halteres can precisely oscillate up and down with a single power muscle.

Driving hemolymph in the wing

An insect wing is made up of thin membranes and flexible, tubular veins, which can allow the wing to bend, twist, and fold. Within these interconnected veins, nerves and tracheae branch, while hemolymph circulates, hydrating embedded resilin and other wing tissues (Arnold 1964; Pass 2018; Salcedo and Socha 2020; Salcedo et al. 2023). Hemolymph circulation in the adult wing contributes to functioning immune responses (e.g., clotting), supplying organs on the wing (i.e., tympana or pheromone-producing), unfolding wings in some beetles, and unique coloration (Sun et al. 2014; Tsai et al. 2020; Nishida et al. 2023). Wing expansion, during ecdysis, is a critical moment in an insect's metamorphosis that requires efficient and productive use of the hemodynamic system, inflation of the tracheal network, and coordinated muscular pumping (amidst all the concurrent hormonal and neuronal activation). Despite its importance, how wing circulation and active hemolymph hydraulics contribute towards flight and unfurling of the wing, are often neglected in studies of wing properties and flight mechanics. In the last decade, there has been greater attention to the importance of circulation and its evolution with respect to the flight motor system and wing hinge components (Hillyer and Pass 2020; Rajabi et al. 2020).

Hemolymph within a flapping wing circulates more quickly than a wing at rest (Wang et al. 2020; Salcedo et al. 2023) and hydrated wings do positively (and somewhat passively) influence wing damping (Lietz et al. 2021). In dragonflies and other insects, the pterostigma, a thickened portion of the leading edge of the wing and a sinus for hemolymph, acts as an inertial regulator, removing instabilities like flutter (Arnold 1963; Norberg 1972). Hemolymph is likely to move when flexion lines in wings fold, are bent, or twisted. This movement of hemolymph may cause some shift in mass that is important for general flight mechanics. When we consider that desiccation of insect cuticle dramatically changes its mechanical properties (Dirks and Taylor 2012a), accrued wing damage over the life of a winged insect is tied to not only to a wing's venation pattern and resistance to breaking (Dirks and Taylor 2012b; Rajabi et al. 2017), but also to how active hemolymph circulates (Rajabi et al. 2020).

To circulate hemolymph throughout the wings, body, and other high-demand sensory organs (i.e., antennae), insects require accessory pulsatile organs to push or pull hemolymph, in addition to their main pumping organ, the dorsal vessel (Pass 1998). These "thoracic wing hearts“” function as suction pumps, pulsing asynchronously with the dorsal vessel, often at higher frequencies (Chintapalli and Hillyer 2016; Pass 2018; Salcedo et al. 2023). Between hemi- and holometabolous insects, we see divergence in pump type correlated with evolution and enlargement of the power-producing dorsal longitudinal flight muscles in the thorax (Krenn and Pass 1994). Further comparative work on how these pumps produce flow, function in active flight, and compensate for damage could reveal important evolutionary modifications of the circulatory system with respect to parameters such as varied venation, flapping frequencies, and body size.

Environmental influences on flight performance

Insects have evolved to fly in dynamic environments, requiring the short- or long-term modulation of their physiology and behavior. On geological timescales (on the order of tens or hundreds of millions of years), changing atmospheric oxygen and temperature conditions, as well as competition or predation by coeval vertebrates, may have affected the kinematic landscape of insect flight in drastic ways (Kaiser et al. 2007; Harrison et al. 2010; Clapham and Karr 2012; Ellers et al. 2024). Moreover, on physiological timescales during flight, insects experience variation in abiotic (*i.e.*, temperature, humidity, wind, rain, and solar radiation) and biotic (*i.e.*, nutrition, mate availability, and predators) factors simultaneously. For example, many small insects are ectothermic during flight and rely on air temperature and solar radiation to warm their muscles enough to be able to fly (Watt 1968; Advani et al. 2019). The reliance on external sources of heat limits insect activity to parts of the day that are not too cold or too hot with potential consequences for geographical range limits (Kingsolver and Watt 1983; Keena 2018; Sun et al. 2020). In contrast, many large, flying insects are endothermic during flight, potentially allowing for flight at air temperatures that could limit their ectothermic counterparts (Heinrich 1993). High air temperatures can become a problem for endothermic fliers, as the addition of metabolic heat to the already significant effects of air temperature and solar load threatens these animals with overheating and desiccation (Johnson et al. 2023a; Glass et al. 2024). While these statements about the value and cost of endothermy seem obvious, they are actually hypotheses that need to be tested. For example, flexibility in the thermal performance of some ectotherms may allow them to fly at similar cool air temperatures as endotherms.

It is clear that only reasonably large insects (> 20 mg) can be endothermic (Duell et al. 2022; Johnson et al. 2023b), but many other aspects of how size affects insect thermal biology remain poorly understood. Small-bodied insects have relatively more surface area compared to body volume than larger counterparts, resulting in rapid heat and water flux. Therefore, in cooler environments, a small insect basking first thing in the morning will warm up faster than a larger one with the same physical surface properties, and the body temperature of a larger insect will vary less in response to brief environmental changes due to its greater thermal mass. However, many questions remain. Are very small insects less affected by thermal variation due to their reduced need to generate lift within relatively more viscous media (Byrne et al. 1988; Blackmer and Byrne 1993; Duell et al. 2022)? Are smaller insects generally more water-limited during flight? Does body size or endothermy affect cuticular reflectance or insulation? Are large insects more limited by heat?

Historically, researchers have independently studied factors affecting flight performance, most often in laboratory settings, to avoid the confounding effects of interacting variables. Temperature and oxygen limitations on flight have been two of the most heavily studied variables, while the effects of humidity, wind speed, and solar radiation have been largely ignored. However, the natural world is constantly fluctuating, and a change in one variable is often associated with changes in one or more other environmental variables. To accurately predict how insects will fare in a changing world, we must shift our focus from investigating single-variable effects to the effects of multiple, dynamic environmental factors and their potential negative, additive, and/or synergistic effects on insect flight performance. For example, insects flying in hot, dry environments may be limited by dehydration, and not by heat (Johnson et al. 2023a; Glass et al. 2024), suggesting that insects could become further limited in their flight activity time. The interactive effects of air temperature and relative humidity may be context-dependent. Many flying insects can actively or passively cool themselves through evaporation (Nicolson and Louw 1982; Prange 1996; Roberts and Harrison 1999; Johnson et al. 2022; Glass et al. 2024), but to date, no study has examined the interactions between humidity, evaporative cooling, and body temperatures for flying insects. We can predict that large, flying insects reliant on evaporative cooling will likely have higher body temperatures when flying in a humid environment compared with a dry one, as high humidities impede evaporation. But how strong an effect this might be is unclear. Understanding such interactions among abiotic factors and among abiotic and biotic factors will be crucial to better predict consequences of climate change for insect flight.

Integrating sensory information to control flight behavior

In the last century, studies of insect flight—particularly in locusts—played a critical role in the discovery of important general phenomena in neuroscience. For example, Don Wilson's work on the neuronal basis of the flight rhythm in locusts provided unambiguous evidence for the existence of central pattern generators within nervous systems (Wilson 1961), a controversial topic at the time (Edwards 2006). Experiments in many laboratories on central and peripheral flight circuitry identified the critical role of neuromodulators such as octopamine (the invertebrate analog of noradrenaline) in modulating and coordinating the activity of neural circuits during behavior, providing an early mechanistic explanation for behavioral state changes within the brain (Orchard et al. 1993). Other studies demonstrated how sensory information from different modalities can be fused together in descending neurons to provide appropriate commands to the local circuits that regulate motor output (Reichardt and Rowell 1985; Reichert 1989; Burrows 1996). During the same era when locusts served as an important general model for neuroscience, studies of in blowflies, dragonflies, and hoverflies (Olberg 1981; O'Carroll 1993; Borst et al. 2010; Nordström 2012) provided critical insight into visual processing, whereas experiments in moths were essential for understanding olfaction during plume tracking (Vickers et al. 1998). Thus, at one time, the study of insect flight was a vibrant part of mainstream neurobiology, and important discoveries emerged from a variety of different species.

For a variety of reasons, the landscape of insect flight neurobiology has changed quite drastically over the last few decades. With more and more research focused on the fruit fly, *Drosophila melanogaster*, a rapidly expanding toolkit of genetic tools for recording from and manipulating the activity of specific cells makes it possible to overcome experimental barriers that faced earlier generations of researchers (Venken et al. 2011). This emphasis on *Drosophila* is both a blessing and a curse for the community. On the one hand, new approaches such as optogenetics and connectomics make it possible to routinely perform experiments that appeared nearly impossible just a few years ago. On the other hand, the over-emphasis on one particular species diminishes the general insight that can only arise through a comparative analysis or requires the study of animals with different behavioral repertoires than those of fruit flies. Fortunately, there are signs that the discoveries emerging from *Drosophila* may serve a role in fostering and empowering research in other species by providing a beachhead of knowledge and an experimental workflow that exploits the deep homologies that exist across species.

There is perhaps no better example of how productive the synergy between *Drosophila* research and studies in other species is than recent advances in our understanding of the central complex, a system of ancient midline brain regions that are essential to many aspects of flight behavior, including navigation (Strausfeld 2012; Honkanen et al. 2019). Experiments using genetically encoded calcium indicators in flies led to the discovery of a ring-attractor network in the ellipsoid body that functions as a compass system, analogous to head direction cells in the mammalian hippocampus (Turner-Evans and Jayaraman 2016; Green et al. 2017; Kim et al. 2017). Very recently, elegant evidence has emerged that another unpaired neuropil within the central complex, the fan-shaped body, contains arrays of cells that are capable of encoding both the magnitude and direction of vectors, such as translational velocity or apparent wind direction (Lyu et al. 2022; Matheson et al. 2022), and that this information can be used to create steering information that orients the animal toward a specific goal (Mussells et al. 2024; Westeinde et al. 2024). The discovery of a region in the brain that can store and manipulate vectors is quite profound because it provides a mechanism by which insects might compute state variables that are critical for flight-related behaviors such as maintaining a constant heading, celestial navigation, ground speed regulation, anemotaxis, altitude regulation, and path integration (van Breugel et al. 2022; Stupski and Breugel 2023). Whereas the key experimental studies on the central complex required the genetic approaches currently restricted to *Drosophila*, comparative studies indicate an astonishing homology of the underlying circuitry across species (Honkanen et al. 2019; Beetz and El Jundi 2023). This allows for a productive synthesis of knowledge—including the generation of neuronally constrained computational models of behavior (Stone et al. 2017; Mitchell et al. 2023)—using anatomical and physiological data from other species that utilize the circuitry to execute more exotic behaviors such as path integration to and from a hive by bees or time-compensated celestial navigation by migratory butterflies. What is emerging is a fascinating picture of how an ancient brain structure—likely dating back to before the emergence of flight—has been co-opted and modified by evolution to perform different navigational tasks.

As indicated above, until recently, the bulk of our knowledge on the flight control circuitry in insects was based on work in locusts and other large orthopteran species that use synchronous flight muscles to power their wing motion. This is not a coincidence. The fact that the wingbeat cycle of locusts is based on a central pattern generator and proprioceptive reflexes makes it possible to aggressively dissect the thorax to provide access to the ventral nerve cord without completely disrupting the underlying flight rhythm (Robertson 2020). Indeed, even a completely isolated thoracic ganglion can generate a rudimentary flight rhythm (Stevenson and Kutsch 1987), an unambiguous demonstration that the nervous system contains central pattern generators. However, the critical role of thorax and wing mechanics in the resonant mechanisms underlying the flight motor of asynchronous insects (e.g., Coleoptera, Hymenoptera, Diptera) has long hindered progress on understanding the flight circuits of these diverse and ecologically important groups. Even minor manipulations required for electrophysiological access to the ventral nerve cord usually disrupt the thorax mechanics so as to render the flight system inoperable. Although it has been possible to gain much insight by recording from flight muscles in asynchronous fliers using electromyography (EMGs) (Harcombe and Wyman 1977; Hürkey et al. 2023), the critical premotor interneurons have remained almost entirely inaccessible. This is one domain in which the genetic tools recently available in *Drosophila* are filling a fundamental gap in our knowledge of flight control circuitry (Ehrhardt et al. 2023; Cheong et al. 2023b).

Perhaps the most compelling example of the utility of the genetic tools available in *Drosophila* for studies of flight motor circuitry concerns the function of descending neurons, a critical class of several hundred interneurons with cell bodies and dendrites in the brain and terminals in the ventral nerve cord (Hsu and Bhandawat 2016; Namiki et al. 2018). These large cells constitute a critical information bottleneck linking sensory structures on the head (e.g., eyes, antennae, and ocelli) to flight motor neurons and premotor networks in the ventral nerve cord. Access to cell-specific genetic driver lines for a vast number of descending neurons, along with data from connectomes on their inputs and outputs, is providing novel insight into the function of specific neurons in many features of flight behavior, including takeoff (von Reyn et al. 2014; von Reyn et al. 2017; Ache et al. 2019), steering (Suver et al. 2016; Namiki et al. 2022), collision avoidance (Kim et al. 2023), spontaneous turns (Ros et al. 2024), and landing (Ache et al. 2019). Among the advantages of the genetic approach to studying the descending neurons are experimental access to very small neurons (that would never be accessible by traditional sharp electrode recording) and optogenetic techniques for experimental activation and silencing in intact animals (Simpson 2024). Recent successes employing these approaches include the discovery of an elegant and remarkably versatile circuit that regulates takeoff in flies. It has long been known that many insects possess a pair of giant descending neurons (the so-called "giant fiber") that mediate fast, uncontrolled escape responses (Trimarchi and Schneiderman 1995). The nervous system also contains a parallel pathway that translates visual information into a set of leg movements prior to takeoff that can allow the fly to deliberately launch itself away from a looming threat (Dombrovski et al. 2023)—providing a neurobiological answer to the question, "why are flies so hard to swat?."

Another example concerns the neuronal basis of a prominent search motif exhibited by flies, in which they execute very straight flight trajectories interspersed with rapid turns called body saccades. Studies of the descending neurons involved in flight control suggest that, although deceptively simple, straight stable flight actually requires a large number of descending neurons operating via a population code (Namiki et al. 2022), whereas the body saccades are controlled by a relatively simple system or just four descending neurons organized into two couplets, each consisting of co-active excitatory and inhibitory cells that function effectively as one single command unit (Ros et al. 2024). The best explanation for why straight flight requires a much larger number of descending neurons than the body saccades is that non-linearities in the aerodynamics of flapping flight require extraordinary precision in order to perfectly trim wing motion and thus balance forces and zero out torques during straight flight. In addition to this precision dictated by the underlying physics, the motor system also requires a large dynamic range in case the left and right wings must flap differently to accommodate morphological asymmetries due to either wing damage or developmental abnormalities (Muijres et al. 2017). Providing this combination of dynamic range and precision is the most likely explanation for the relatively large number of descending neurons necessary for straight flight. Although body saccades are impressive acrobatic maneuvers, their variability from event to event (Muijres et al. 2015) suggests a lack of precision that is consistent with the smaller number of descending neurons that are required for their execution.

Whereas most research to date on flight control circuitry using genetic tools has focused on descending and motor neurons, the same approaches are becoming available to investigate local interneurons and sensory neurons—two cell classes that have been particularly difficult to record and manipulate in flies and other insects with an asynchronous flight motor. As was the case with descending neurons, these investigations will be facilitated by the availability of sparse genetic driver lines, which may be screened using both activation and silencing techniques. In addition, data from several connectomes suggest much functional structure within ventral nerve cord circuits, which link together the activation of specific flight motor neurons that play synergistic roles in flight control (Ehrhardt et al. 2023; Lesser et al. 2023; Cheong et al. 2023b). A key challenge for future studies of flight circuitry will be in determining how descending information from the eyes, antennae, and ocelli is integrated with local mechanosensory feedback from the wings (and halteres in flies) to generate appropriate commands to flight motor neurons such that they fire at the correct time in each wingbeat cycle. In addition, the emerging connectome has brought attention to the importance of the large populations of ascending neurons, which serve as a critical conduit for providing sensory feedback and efference copy from the ventral nerve cord to the brain (Cheong et al. 2023a).

Outlook and potential solutions to methodological barriers

As discussed above, an enormous and rapid amount of progress has been made within the last several decades to address long-standing questions about the developmental origins of insect wings, the physiological and biomechanical determinants of insect flight performance, and the neurological and sensory processes controlling flight. Integrative research on the physiology and biomechanics of insect has also had wide-reaching broader impacts, informing policy makers (e.g., Marden and Allen 2002; Fisher et al. 2023; Siviter et al. 2023), agricultural practices (e.g., Cease et al. 2015), and bio-inspired design principles applied by engineers (e.g., Franceschini et al. 2007; Sato et al. 2009; Ma et al. 2013; Phan and Park 2020). However, recent progress has raised new questions, and many pressing gaps remain, particularly in our understanding of how complex interactions among genetic, morphological, physiological, and ecological factors shape both past and present evolutionary dynamics of insect flight. Many of these gaps in knowledge exist in part because of barriers posed by technical limitations to studying insect flight. Moving forward will surely require innovative and interdisciplinary solutions to these challenges, and we briefly discuss potential avenues for methodological development, which we think should be prioritized.

We are currently constrained in our ability to study broadscale cross-species or cross-population patterns and evolutionary trends because much of our current knowledge base and research is constrained to a few model insect taxa. For example, the overwhelming majority of data on the gene regulatory networks governing wing development (Tripathi and Irvine 2022) and neurobiological function of the flight system comes from *Drosophila melanogaster*, which exhibits many highly derived aspects of development and flight behavior. One key to addressing this shortcoming is to prioritize developing high-quality genomes and transcriptomes using long-read sequencing technology, for more non-model insects. These efforts will help make a suite of additional cutting-edge molecular genetic tools (e.g., in situ hybridization/HCR, antibody staining, single-cell RNA-seq, RNAi, CRISPR-Cas9 genome editing, transgenics for reporter assay, overexpression, lineage tracing, and chromatin profiling methods) more tractable to employ, and open up new avenues for research on the evolutionary diversification of flight using functional genomic approaches. Additionally, the improved resolution of the insect phylogeny (Misof et al. 2014) should be better leveraged to focus efforts on studying specific members of major taxa in closely related clades to act as representatives where major evolutionary transitions in flight machinery took place (Fig. 3). This approach was central to providing the recent insights into the evolutionary transition in flight muscle from asynchrony to synchrony in Lepidopteran discussed above (Gau et al. 2023), indicative of the great value and importance of studying insect flight in a phylogenetic context.

Additionally, the large amount of variation among insects in flight propensity and mode of flight poses a major challenge to defining and measuring flight performance broadly across taxa. In particular, the relatively small body sizes and high speeds at which insects fly make studying diverse flight behaviors, including maximum flight speeds, agility, maneuverability, and endurance very challenging. Thus, large taxonomic biases in the physiological and biomechanical literature on insect flight arise simply from our constrained ability to measure flight most readily among larger bodied and relatively slow flying insects. The development and use of high-speed 3D video camera technology and sophisticated analysis tools, which enable tracking of insect flight at small spatial scales with high resolution and provide accurate measures of short-term acceleration, was instrumental in advancing our ability to study the biomechanical basis of flight performance (Fabian et al. 2024). However, a complementary toolkit for physiologists to monitor, measure, and manipulate the physiological state of insects in flight is lacking. Organismal flight metabolic rates are available for a relatively broad range taxa and have served as a critical foundation for our understanding of flight energetics and physiological costs that constrain flight performance. To move the field forward, there are clear needs for improved, high-throughput methods for better assessing other aspects of the insect physiological state during flight, including parameters such as muscle activation, force generation, substrate and adenylate concentrations, gas concentrations, and oxidative damage. Development of these tools may require partnerships with engineers but would be very useful for addressing open questions about how insect maximal flight performance is related to wing morphology and kinematics, the physiological causes of short- and long-term fatigue, and environmental effects on performance limits.

Finally, to better predict and explain the evolutionary consequences of variation in flight performance, there is a critical need to investigate how flight performance of insects measured in the laboratory setting translates into the field. In natural settings, most insects only fly intermittently or in specific environmental conditions or life stages, making complex relationships between lab-based measures of flight performance and behavior of individuals in natural settings likely. For example, whereas female Glanville fritillary butterflies show a positive relationship between flight metabolic rate and the distance covered in the field (Niitepõld et al. 2009), the relationship appears to be the opposite in males, probably due to male flight being more oriented towards mate location and within-sex competition (Niitepõld et al. 2011). New advances in GPS monitoring, radio transmitters, and telemetry are excitedly improving our ability to track free-flying insects over large spatial scales (Dudley and Srygley 1994; Osborne et al. 1999; Cant et al. 2005; Dudley and Srygley 2008; Kőrösi et al. 2008; Ovaskainen et al. 2008; Chapman et al. 2011; Knight et al. 2019; Menz et al. 2022). Increased use and application of these technologies will help to fill major gaps in our understanding of how far and frequently insects fly in nature. There is also great potential for combining lab and field datasets on flight to develop ecological and evolutionary models predicting how insect flight patterns will be affected by changing climatic conditions.

Conclusions

Understanding the diversity and evolutionary dynamics of flight strategies requires an integrative approach, focused on linking genes up to organismal flight performance and fitness in variable environments. This symposium showcased the diversity in flight strategies that exists across the insect phylogeny and has illustrated the ways in which the mechanistic underpinnings of flight combine with the environmental conditions to determine the targets of selection. Clearly, many metabolic properties of the muscles are associated with the flight performance of species and individuals, but the diversity in performance (e.g., endurance, maneuverability, load-lifting, and life-history strategies), physiological properties (e.g., synchronous-asynchronous, endothermic capacity, metabolic fuel, and oxygen delivery), and the aerodynamic regime occupied by species can influence the specific physiological targets that evolutionary mechanisms may act on.

Trade-offs are key to understanding the constraints shaping the evolution of flight, and the study of insect flight is advancing our understanding of the mechanisms and evolutionary consequences of these trade-offs. In this symposium, we have considered that trade-offs occur due to resource allocation or acquisition constraints (e.g., dispersal-reproduction trade-offs), functional conflicts in the physiological and biomechanical mechanisms (e.g., maneuverability vs. stability, burst performance vs. endurance), and due to variation in the ecological context (e.g., thermal and hygric conditions). Sophisticated environmental sensing mechanisms and physiological integration enables flight strategies to be finely tuned to environmental variation to match flight performance to environmental conditions. Further study of the underlying mechanisms of this integration of environmental information and coupling it to physiological and biomechanical outputs promises to provide us with important insights into how insects will respond to environmental change.

Acknowledgments

We would like to thank all of the participants who attended the insect flight workshop during the Society for Integrative and Comparative Biology (SICB) 2024 annual meeting for their intellectual contributions and discussion on outstanding questions in the field, and feedback from two anonymous reviewers that helped improve the quality of this manuscript.

Funding

This work, symposium, and workshop was supported by the Society for Integrative and Comparative Biology Division of Ecology and Evolution, Division of Comparative Physiology and Biochemistry, Division of Evolutionary Developmental Biology, Division of Invertebrate Zoology, Division of Neurobiology, Neuroethology, and Sensory Biology; the American Microscopy Society; the Company of Biologists [Scientific meeting grant EA774]; the National Science Foundation [IOS-2326924, IOS-2100858 to S.S., DBI-2305817 to K.D.D., DBI-2109468 to L.A.T., IOS-2319792 to C.M.W.]; Air Force Office of Scientific Research [MURI grant FA9550-22-1-0315 to S.S.]; Natural Sciences and Engineering Research Council of Canada [Postdoctoral Fellowship to J.E.L.]; the National Institute of General Medical Sciences of the National Institutes of Health [R35GM144001 supported K.D.D.]; and the United States Department of Agricultrue National Institute of Food and Agriculture [Grant #2021-08373 to M.K.S.].

Conflict of interest

The authors have no conflicts of interest to declare.

Data availability

There is no data associated with this manuscript.

References

Ache JM, Namiki S, Lee A, Branson K, Card GM. 2019. State-dependent decoupling of sensory and motor circuits underlies behavioral flexibility in *Drosophila*. Nat Neurosci 22:1132–9. https://doi.org/10.1038/s41593-019-0413-4

Advani NK, Parmesan C, Singer MC. 2019. Takeoff temperatures in *Melitaea cinxia* butterflies from latitudinal and elevational range limits: a potential adaptation to solar irradiance. Ecol Entomol 44:389–96. https://doi.org/10.1111/een.12714

Aiello BR, Tan M, Bin Sikandar U, Alvey AJ, Bhinderwala B, Kimball KC, Barber JR, Hamilton CA, Kawahara AY, Sponberg S. 2021. Adaptive shifts underlie the divergence in wing morphology in *Bombycoid* moths. Proc R Soc B: Biol Sci 288:20210677. https://doi.org./10.1098/rspb.2021.0677

Altaratz M, Applebaum SW, Richard DS, Gilbert LI, Segal D. 1991. Regulation of juvenile hormone synthesis in wild-type and apterous mutant *Drosophila*. Mol Cell Endocrinol 81:205–16. https://doi.org/10.1016/0303-7207(91)90219-I

Andersen NM. 1993. The evolution of wing polymorphism in water striders (Gerridae): a phylogenetic approach. Oikos 67:433–43. https://doi.org/10.2307/3545355

Aranha MM, Herrmann D, Cachitas H, Neto-Silva RM, Dias S, Vasconcelos ML. 2017. Apterous brain neurons control receptivity to male courtship in *Drosophila melanogaster* females. Sci Rep 7:46242. https://doi.org/10.1038/srep46242

Arnold JW. 1963. A note on the pterostigma in insects. Can Entomol 95:13–6. https://doi.org/10.4039/Ent9513-1

Arnold JW. 1964. Blood circulation in insect wings. Mem Entomol Soc Can 96:5–60. https://doi.org/10.4039/entm9638fv

Askew GN, Tregear RT, Ellington CP. 2010. The scaling of myofibrillar actomyosin ATPase activity in *Apid* bee flight muscle in relation to hovering flight energetics. J Exp Biol 213:1195–206. https://doi.org/10.1242/jeb.034330

Averof M, Cohen SM. 1997. Evolutionary origin of insect wings from ancestral gills. Nature 385:627–30. https://doi.org/10.1038/385627a0

Bank S, Bradler S. 2022. A second view on the evolution of flight in stick and leaf insects (Phasmatodea). BMC Ecol Evol 22:62. https://doi.org/10.1186/s12862-022-02018-5

Beenakkers AMT, Van Der Horst DJ, Van Marrewijk WJA. 1984. Insect flight muscle metabolism. Insect Biochem 14:243–60. https://doi.org/10.1016/0020-1790(84)90057-X

Beetz MJ, El Jundi B. 2023. The influence of stimulus history on directional coding in the monarch butterfly brain. J Comp Physiol A 209:663–77. https://doi.org/10.1007/s00359-023-01633-x

Bejan A, Marden JH. 2006. Unifying constructal theory for scale effects in running, swimming and flying. J Exp Biol 209:238–48. https://doi.org/10.1242/jeb.01974

Bernard F, Lalouette A, Gullaud M, Jeantet AY, Cossard R, Zider A, Ferveur JF, Silber J. 2003. Control of apterous by vestigial drives indirect flight muscle development in *Drosophila*. Dev Biol 260:391–403. https://doi.org/10.1016/S0012-1606(03)00255-0

Billardon F, Darveau C-A. 2019. Flight energetics, caste dimorphism and scaling properties in the bumblebee, *Bombus impatiens*. J Exp Biol 222: jeb187807. https://doi.org/10.1242/jeb.187807

Blackmer JL, Byrne DN. 1993. Flight behaviour of *Bemisia tabaci* in a vertical flight chamber: effect of time of day, sex, age and host quality. Physiol Entomol 18:223–32. https://doi.org/10.1111/j.1365-3032.1993.tb00592.x

Boettiger EG, Furshpan E. 1952. The mechanics of flight movements in diptera. Biol Bull 102:200–11. https://doi.org/10.2307/1538368

Borst A, Haag J, Reiff DF. 2010. Fly motion vision. Annu Rev Neurosci 33:49–70. https://doi.org/10.1146/annurev-neuro-060909-153155

Bruce HS, Patel NH. 2020. Knockout of crustacean leg patterning genes suggests that insect wings and body walls evolved from ancient leg segments. Nat Ecol Evol 4:1703–12. https://doi.org/10.1038/s41559-020-01349-0

Bruce HS, Patel NH. 2022. Cryptic Persistence of truncated abdominal legs in insects enabled diverse outgrowths with novel functions. Preprints 202212068. https://www.preprints.org/manuscript/202212.0268/v3 (Accessed 7/20/2024)

Burrows M. 1996. The neurobiology of an insect brain. New York (NY): Oxford University Press. https://doi.org/10.1093/acprof:oso/9780198523444.001.0001

Byrne DN, Buchmann SL, Spangler HG. 1988. Relationship between wing loading, wingbeat frequency and body mass in homopterous insects. J Exp Biol 135:9–23. https://doi.org/10.1242/jeb.135.1.9

Cant ET, Smith AD, Reynolds DR, Osborne JL. 2005. Tracking butterfly flight paths across the landscape with harmonic radar. Proc R Soc B Biol Sci 272:785–90. https://doi.org/10.1098/rspb.2004.3002

Cao T, Jin J-P. 2020. Evolution of flight muscle contractility and energetic efficiency. Front Physiol 11:1038. https://doi.org/10.3389/fphys.2020.01038

Casey C, Heveran C, Jankauski M. 2023. Experimental studies suggest differences in the distribution of thorax elasticity between insects with synchronous and asynchronous musculature. J R Soc Interface 20:20230029. https://doi.org/10.1098/rsif.2023.0029

Casey TM. 1981. A comparison of mechanical and energetic estimates of flight cost for hovering sphinx moths. J Exp Biol 91:117–29. https://doi.org/10.1242/jeb.91.1.117

Casey TM, May ML, Morgan KR. 1985. Flight energetics of euglossine bees in relation to morphology and wing stroke frequency. J Exp Biol 116:271–89. https://doi.org/10.1242/jeb.116.1.271

Cease AJ, Elser JJ, Fenichel EP, Hadrich JC, Harrison JF, Robinson BE. 2015. Living with locusts: connecting soil nitrogen, locust outbreaks, livelihoods, and livestock markets. Bioscience 65:551–8. https://doi.org/10.1093/biosci/biv048

Chapman JW, Drake VA, Reynolds DR. 2011. Recent insights from radar studies of insect flight. Annu Rev Entomol 56:337–56. https://doi.org/10.1146/annurev-ento-120709-144820

Cheong HSJ, Boone KN, Bennett MM, Salman F, Ralston JD, Hatch K, Allen RF, Phelps AM, Cook AP, Phelps JS et al. 2023a. Organization of an ascending circuit that conveys flight motor state. *bioRxiv*. 2023.2006.2007.544074. https://doi.org/10.1101/2023.06.07.544074 (Accessed 7/20/2024)

Cheong HSJ, Eichler K, Stürner T, Asinof SK, Champion AS, Marin EC, Oram TB, Sumathipala M, Venkatasubramanian L, Namiki S et al. 2023b. Transforming descending input into behavior: the organization of premotor circuits in the *Drosophila* male adult nerve cord connectome. *bioRxiv*.2023.2006.2007.543976. https://doi.org.10.1101/2023.06.07.543976 (Accessed 7/20/2024)

Chintapalli RTV, Hillyer JF. 2016. Hemolymph circulation in insect flight appendages: physiology of the wing heart and circulatory flow in the wings of the mosquito *Anopheles gambiae*. J Exp Biol 219:3945–51. https://doi.org/10.1242/jeb.148254

Cibotti S, Saum PJ, Myrick AJ, Schilder RJ, Ali JG. 2024. Divergent impacts of the neonicotinoid insecticide, clothianidin, on flight performance metrics in two species of migratory butterflies. Conser Physiol 12:coae002. https://doi.org/10.1093/conphys/coae002

Clapham ME, Karr JA. 2012. Environmental and biotic controls on the evolutionary history of insect body size. Proc Natl Acad Sci USA 109:10927–30. https://doi.org/10.1073/pnas.1204026109

Clark-Hachtel CM, Linz DM, Tomoyasu Y. 2013. Insights into insect wing origin provided by functional analysis of *vestigial* in the red flour beetle, *Tribolium castaneum*. Proc Nat Acad Sci 110:16951–6. https://doi.org/10.1073/pnas.1304332110

Clark-Hachtel CM, Tomoyasu Y. 2020. Two sets of candidate crustacean wing homologues and their implication for the origin of insect wings. Nat Ecol Evol 4:1694–702. https://doi.org/10.1038/s41559-020-1257-8

Cohen B, Mcguffin ME, Pfeifle C, Segal D, Cohen SM. 1992. Apterous, a gene required for imaginal disc development in *Drosophila* encodes a member of the lim family of developmental regulatory proteins. Genes Dev 6:715–29. https://doi.org/10.1101/gad.6.5.715

Crampton G. 1916. The phylogenetic origin and the nature of the wings of insects according to the paranotal theory. J N Y Entomol Soc 24:1–39.

Darveau C-A, Hochachka PW, Roubik DW, Suarez RK. 2005a. Allometric scaling of flight energetics in orchid bees: evolution of flux capacities and flux rates. J Exp Biol 208:3593–602. https://doi.org/10.1242/jeb.01777

Darveau C-A, Hochachka PW, Welch KC, Jr, Roubik DW, Suarez RK. 2005b. Allometric scaling of flight energetics in panamanian orchid bees: a comparative phylogenetic approach. J Exp Biol 208:3581–91. https://doir.org/10.1242/jeb.01776

Darwin C. 1859. The origin of species by means of natural selection: or, the preservation of favored races in the struggle for life. London: Collector's Library.

Deakin MAB. 2010. Formulae for insect wingbeat frequency. J Insect Sci 10:1–9. https://doi.org/10.1673/031.010.9601

Deem KD, Brisson JA. 2024. Problems with paralogs: the promise and challenges of gene duplicates in evo-devo research. Integr Comp Biol 2:icae009. https://doi.org/10.1093/icb/icae009

Deora T, Gundiah N, Sane SP. 2017. Mechanics of the thorax in flies. J Exp Biol 220:1382–95. https://doi.org/10.1242/jeb.128363

Deora T, Sane SS, Sane SP. 2021. Wings and halteres act as coupled dual oscillators in flies. eLife 10:e53824. https://doi.org/10.7554/eLife.53824

Dickinson MH, Lehmann F-O, Sane SP. 1999. Wing rotation and the aerodynamic basis of insect flight. Science 284:1954–60. https://doi.org/10.1126/science.284.5422.1954

Dirks J-H, Taylor D. 2012a. Fracture toughness of locust cuticle. J Exp Biol 215:1502–8. https://doi.org/10.1242/jeb.068221

Dirks J-H, Taylor D. 2012b. Veins improve fracture toughness of insect wings. PLoS One 7:e43411. https://doi.org/10.1371/journal.pone.0043411

Dollo L. 1893. The laws of evolution. Bull Soc Bel Geol Paleontol 7:164–6.

Dombrovski M, Peek MY, Park J-Y, Vaccari A, Sumathipala M, Morrow C, Breads P, Zhao A, Kurmangaliyev YZ, Sanfilippo P et al. 2023. Synaptic gradients transform object location to action. Nature 613:534–42. https://doi.org/10.1038/s41586-022-05562-8

Du B, Ding D, Ma C, Guo W, Kang L. 2022. Locust density shapes energy metabolism and oxidative stress resulting in divergence of flight traits. Proc Nat Acad Sci 119:e2115753118. https://doi.org/10.1073/pnas.2115753118

Ducatez S, Legrand D, Chaput-Bardy A, Stevens VM, Fréville H, Baguette M. 2012. Inter-individual variation in movement: is there a mobility syndrome in the large white butterfly *Pieris brassicae*? Ecol Entomol 37:377–85. https://doi.org/10.1111/j.1365-2311.2012.01375.x

Dudley R. 1995. Extraordinary flight performance of orchid bees (Apidae: Euglossini) hovering in heliox (80% He/20% O_2). J Exp Biol 198:1065–70. https://doi.org/10.1242/jeb.198.4.1065

Dudley R. 2000. The Biomechanics of Insect Flight: Form, Function, Evolution. Princeton (NJ): Princeton University Press.

Dudley R, Byrnes G, Yanoviak SP, Borrell B, Brown RM, Mcguire JA. 2007. Gliding and the functional origins of flight: biomechanical novelty or necessity? Annu Rev Ecol Evol System 38:179–201. https://doi.org/10.1146/annurev.ecolsys.37.091305.110014

Dudley R, Srygley RB. 1994. Flight physiology of neotropical butterflies: allometry of airspeeds during natural free flight. J Exp Biol 191:125–39. https://doi.org/10.1242/jeb.191.1.125

Dudley R, Srygley RB. 2008. Airspeed adjustment and lipid reserves in migratory neotropical butterflies. Funct Ecol 22:264–70. https://doi.org/10.1111/j.1365-2435.2007.01364.x

Dudley R, Yanoviak SP. 2011. Animal aloft: the origins of aerial behavior and flight. Integr Comp Biol 51:926–36. https://doi.org/10.1093/icb/icr002

Duell ME, Klok CJ, Roubik DW, Harrison JF. 2022. Size-dependent scaling of stingless bee flight metabolism reveals an energetic benefit to small body size. Integr Comp Biol 62:1429–38. https://doi.org/10.1093/icb/icac131

Edwards JS. 2006. The central nervous control of insect flight. J Exp Biol 209:4411–3. https://doi.org.10.1242/jeb.02592

Ehrhardt E, Whitehead SC, Namiki S, Minegishi R, Siwanowicz I, Feng K, Otsuna H, Team FP, Meissner GW, Stern D et al. 2023. Single-cell type analysis of wing premotor circuits in the ventral nerve cord of *Drosophila melanogaster*. *bioRxiv*.2023.2005.2031.542897. https://doi.org/10.1101/2023.05.31.542897

Ellers O, Gordon CM, Hukill MT, Kukaj A, Cannell A, Nel A. 2024. Induced power scaling alone cannotnot explain griffenfly gigantism. Integr Comp Biol 4:icea046. https://doi.org/10.1093/icb/icae046

Ellington CP. 1984. The aerodynamics of flapping animal flight1. Am Zool 24:95–105. https://doi.org/10.1093/icb/24.1.95

Engel MS, Davis SR, Prokop J. 2013. Insect wings: the evolutionary development of nature's first flyers. In: Minelli A, Boxshall G, Fusco G, editors. Arthropod biology and evolution: molecules, development, morphology. Berlin, Heidelberg: Springer Berlin Heidelberg. p.269–98. https://doi.org/10.1007/978-3-642-36160-9_12

Ennos AR. 1987. A comparative study of the flight mechanism of diptera. J Exp Biol 127:355–72. https://doi.org/10.1242/jeb.127.1.355

Fabian ST, Sondhi Y, Allen PE, Theobald JC, Lin H-T. 2024. Why flying insects gather at artificial light. Nat Commun 15:689. https://doi.org/10.1038/s41467-024-44785-3

Fawcett MM, Parks MC, Tibbetts AE, Swart JS, Richards EM, Vanegas JC, Cenzer M, Crowley L, Simmons WR, Hou WS et al. 2018. Manipulation of insulin signaling phenocopies evolution of a host-associated polyphenism. Nat Commun 9:1699. https://doi.org/10.1038/s41467-018-04102-1

Fisher A, Tadei R, Berenbaum M, Nieh J, Siviter H, Crall J, Glass JR, Muth F, Liao L-H, Traynor K et al. 2023. Breaking the cycle: reforming pesticide regulation to protect pollinators. Bioscience 73:808–13. https://doi.org/10.1093/biosci/biad088

Forni G, Martelossi J, Valero P, Hennemann FH, Conle O, Luchetti A, Mantovani B. 2022. Macroevolutionary analyses provide new evidence of phasmid wings evolution as a reversible process. Syst Biol 71:1471–86. https://doi.org/10.1093/sysbio/syac038

Foster BJ, Mcculloch GA, Vogel MFS, Ingram T, Waters JM. 2021. Anthropogenic evolution in an insect wing polymorphism following widespread deforestation. Biol Lett 17:20210069. https://doi.org/10.1098/rsbl.2021.0069

Franceschini N, Ruffier F, Serres J. 2007. A bio-inspired flying robot sheds light on insect piloting abilities. Curr Biol 17:329–35. https://doi.org/10.1016/j.cub.2006.12.032

Gau J, Gemilere R, Lynch J, Gravish N, Sponberg S, (FM subteam) LDS-VIP, . 2021. Rapid frequency modulation in a resonant system: aerial perturbation recovery in hawkmoths. Proc R Soc B: Biol Sci 288:20210352. https://doi.org/10.1098/rspb.2021.0352

Gau J, Lynch J, Aiello B, Wold E, Gravish N, Sponberg S. 2023. Bridging two insect flight modes in evolution, physiology and robophysics. Nature 622:767–74. https://doi.org/10.1038/s41586-023-06606-3

Gibo DL, Pallett MJ. 1979. Soaring flight of monarch butterflies, *Danaus plexippus* (Lepidoptera: Danaidae), during the late summer migration in southern Ontario. Can J Zool 57:1393–401. https://doi.org/10.1139/z79-180

Glass JR, Burnett NP, Combes SA, Weisman E, Helbling A, Harrison JF. 2024. Flying, nectar-loaded honey bees conserve water and improve heat tolerance by reducing wingbeat frequency and metabolic heat production. Pro Nat Acad Sci 121:e2311025121. https://doi.org/10.1073/pnas.2311025121

Glass JR, Harrison JF. 2022. The thermal performance curve for aerobic metabolism of a flying endotherm. Proc R Soc B Biol Sci 289:20220298. https://doi.org/10.1098/rspb.2022.0298

Goczał J, Beutel RG. 2023. Beetle elytra: evolution, modifications and biological functions. Biol Lett 19:20220559. https://doi.org/10.1098/rsbl.2022.0559

Green J, Adachi A, Shah KK, Hirokawa JD, Magani PS, Maimon G. 2017. A neural circuit architecture for angular integration in *Drosophila*. Nature 546:101–6. https://doi.org/10.1038/nature22343

Greenewalt CH. 1960. The wings of insects and birds as mechanical oscillators. Proc Am Philos Soc 104:605–11.

Greenewalt CH. 1962. Dimensional relationships for flying animals.Smithsonian miscellaneous collections.144:1–46.

Hamilton KGA. 1971. The insect wing, part i. origin and development of wings from notal lobes. J Kans Entomol Soc 44:421–33.

Hanski I, Schulz T, Wong SC, Ahola V, Ruokolainen A, Ojanen SP. 2017. Ecological and genetic basis of metapopulation persistence of the glanville fritillary butterfly in fragmented landscapes. Nat Commun 8:14504. https://doi.org/10.1038/ncomms14504

Harcombe ES, Wyman RJ. 1977. Output pattern generation by *Drosophila* flight motoneurons. J Neurophysiol 40:1066–77. https://doi.org/10.1152/jn.1977.40.5.1066

Harrison JF, Kaiser A, Vandenbrooks JM. 2010. Atmospheric oxygen level and the evolution of insect body size. Proc R Soc B Biol Sci 277:1937–46. https://doi.org/10.1098/rspb.2010.0001

Harrison JF, Nielsen DI, Page RE. 1996. Malate dehydrogenase phenotype, temperature and colony effects on flight metabolic rate in the Honey-bee, *Apis mellifera*. Funct Ecol 10:81–8. https://doi.org/10.2307/2390265

Harrison JF, Roberts SP. 2000. Flight respiration and energetics. Annu Rev Physiol 62:179–205. https://doi.org/10.1146/annurev.physiol.62.1.179

Harrison RG. 1980. Dispersal polymorphisms in insects. Annu Rev Ecol Syst 11:95–118. https://doi.org/10.1146/annurev.es.11.110180.000523

Hedges CP, Wilkinson RT, Devaux JBL, Hickey AJR. 2019. Hymenoptera flight muscle mitochondrial function: increasing metabolic power increases oxidative stress. Comp Biochem Physiol A Mol Integr Physiol 230:115–21. https://doi.org/10.1016/j.cbpa.2019.01.002

Heinrich B. 1971. Temperature regulation of the sphinx moth, *Manduca sexta*: I. flight energetics and body temperature during free and tethered flight. J Exp Biol 54:141–52. https://doi.org/10.1242/jeb.54.1.141

Heinrich B. 1993. The hot-blooded insects: strategies and mechanisms of thermoregulation. Cambridge (MA): Harvard University Press.

Henningsson P, Bomphrey RJ. 2011. Time-varying span efficiency through the wingbeat of desert locusts. J R Soc Interface 9:1177–86. https://doi.org/10.1098/rsif.2011.0749

Hickey T, Devaux J, Rajagopal V, Power A, Crossman D. 2022. Paradoxes of hymenoptera flight muscles, extreme machines. Biophys Rev 14:403–12. https://doi.org/10.1007/s12551-022-00937-7

Hillyer JF, Pass G. 2020. The insect circulatory system: structure, function, and evolution. Annu Rev Entomol 65:121–43. https://doi.org/10.1146/annurev-ento-011019-025003

Honkanen A, Adden A, Da Silva Freitas J, Heinze S. 2019. The insect central complex and the neural basis of navigational strategies. J Exp Biol 222:jeb188854. https://doi.org/10.1242/jeb.188854

Hörnschemeyer T, Willkommen J. 2007. The contribution of flight system characters to the reconstruction of the phylogeny of the pterygota. Arthropod Syst Phylogeny 65:15–23.

Hsu CT, Bhandawat V. 2016. Organization of descending neurons in *Drosophila melanogaster*. Sci Rep 6:20259. https://doi.org/10.1038/srep20259

Hürkey S, Niemeyer N, Schleimer J-H, Ryglewski S, Schreiber S, Duch C. 2023. Gap junctions desynchronize a neural circuit to stabilize insect flight. Nature 618:118–25. https://doi.org/10.1038/s41586-023-06099-0

Iwamoto H. 2011. Structure, function and evolution of insect flight muscle. Biophysics 7:21–8. https://doi.org/10.2142/biophysics.7.21

Jankauski MA. 2020. Measuring the frequency response of the honeybee thorax. Bioinspir Biomim 15:046002. https://doi.org/10.1088/1748-3190/ab835b

Jarzembowski E. 2021. Fossil insects 10 years after the geological conservation review (Great Britain). Palaeoentomology 4:313–318-. https://doi.org.10.11646/PALAEOENTOMOLOGY.4.4.3

Johnson MG, Alvarez K, Harrison JF. 2023a. Water loss, not overheating, limits the activity period of an endothermic sonoran desert bee. Funct Ecol 37:2855–67. https://doi.org/10.1111/1365-2435.14438

Johnson MG, Glass JR, Dillon ME, Harrison JF. 2023b. How will climatic warming affect insect pollinators?In: Harrison JF, editor. Adv insect physiol: Environmental Threats to Pollinator Health and Fitness Cambrige (MA): Academic Press. p.1–115. https://doi.org/10.1016/bs.aiip.2023.01.001

Johnson MG, Glass JR, Harrison JF. 2022. A desert bee thermoregulates with an abdominal convector during flight. J Exp Biol 225:jeb244147. https://doi.org/10.1242/jeb.244147

Josephson RK, Malamud JG, Stokes DR. 2000. Asynchronous muscle: a primer. J Exp Biol 203:2713–22. https://doi.org/10.1242/jeb.203.18.2713

Kaiser A, Klok CJ, Socha JJ, Quinlan MC, Harrison JF. 2007. Increase in tracheal investment with beetle size supports hypothesis of oxygen limitation on insect gigantism. Proc Natl Acad Sci USA 104:13198–203. https://doi.org/10.1073pnas.0611544104

Keena MA. 2018. Factors that influence flight propensity in *Anoplophora Glabripennis* (Coleoptera: Cerambycidae). Environ Entomol 47:1233–41. https://doi.org/10.1093/ee/nvy100

Kim H, Park H, Lee J, Kim AJ. 2023. A visuomotor circuit for evasive flight turns in *Drosophila*. Curr Biol 33:321–335.e326. https://doi.org/10.1016/j.cub.2022.12.014

Kim SS, Rouault H, Druckmann S, Jayaraman V. 2017. Ring attractor dynamics in the *Drosophila* central brain. Science 356:849–53. https://doi.org/10.1126/science.aal4835

Kingsolver JG, Watt WB. 1983. Thermoregulatory strategies in *Colias* butterflies: thermal stress and the limits to adaptation in temporally varying environments. Am Nat 121:32–55. https://doi.org/10.1086/284038

Knight SM, Pitman GM, Flockhart DTT, Norris DR. 2019. Radio-tracking reveals how wind and temperature influence the pace of daytime insect migration. Biol Lett 15:20190327. https://doi.org/10.1098/rsbl.2019.0327

Kobayashi Y, Bruce HS, Terada K, Hirayama A, Oosawa Y, Niikura K, Takami Y. 2022. Paranotal lobes are appendicular in origin: elucidation by Micro-CT analysis of the thoracic muscular system in the larvae of *Carabus insulicola* (Insecta, Coleoptera). Proc Arthropod Embryol Soc Jpn 54:1–9.

Kőrösi Á, Örvössy N, Batáry P, Kövér S, Peregovits L. 2008. Restricted within-habitat movement and time-constrained egg laying of female *Maculinea rebeli* butterflies. Oecologia 156:455–64. https://doi.org/10.1007/s00442-008-0986-1

Krenn HW, Pass G. 1994. Morphological diversity and phylogenetic analysis of wing circulatory organs in insects, part II: holometabola. Zoology 98:7–22.

Kukalova-Peck J. 1978. Origin and evolution of insect wings and their relation to metamorphosis, as documented by the fossil record. J Morphol 156:53–125. https://doi.org/10.1002/jmor.1051560104

Lapina NA, Farisenkov SE, Petrov PN, Polilov AA. 2021. Effects of miniaturization on wing structure and flight mechanics in insects. Entomol Rev 101:1126–41. https://doi.org/10.1134/S001387382108008X

Latreille PA. 1819. Mémoires sur Divers Sujets de L'histoire Naturelle Des Insectes, De Géographie Ancienne et De Chronologie. Paris:Deterville.

Leihy RI, Chown SL. 2020. Wind plays a major but not exclusive role in the prevalence of insect flight loss on remote islands. Proc R Soc B Biol Sci 287:20202121. https://doi.org/10.1098/rspb.2020.2121

Lesser E, Azevedo AW, Phelps JS, Elabbady L, Cook A, Mark B, Kuroda S, Sustar A, Moussa A, Dallmann CJ et al. 2023. Synaptic architecture of leg and wing motor control networks in *Drosophila*. *bioRxiv*.2023.2005.2030.542725. https://doi.org/10.1101/2023.05.30.542725

Li B, Bickel RD, Parker BJ, Saleh Ziabari O, Liu F, Vellichirammal NN, Simon J-C, Stern DL, Brisson JA. 2020. A large genomic insertion containing a duplicated follistatin gene is linked to the pea aphid male wing dimorphism. eLife 9:e50608. https://doi.org/10.7554/eLife.50608

Lietz C, Schaber CF, Gorb SN, Rajabi H. 2021. The damping and structural properties of dragonfly and damselfly wings during dynamic movement. Commun Biol 4:737. https://doi.org/10.1038/s42003-021-02263-2

Lighthill MJ. 1973. On the weis-fogh mechanism of lift generation. J Fluid Mech 60:1–17. https://doi.org/10.1017/S0022112073000017

Lin X, Lavine LC. 2018. Endocrine regulation of a dispersal polymorphism in winged insects: a short review. Curr Opin Insect Sci 25:20–4. https://doi.org/10.1016/j.cois.2017.11.004

Lindsay T, Sustar A, Dickinson M. 2017. The function and organization of the motor system controlling flight maneuvers in flies. Curr Biol 27:345–58. https://doi.org/10.1016/j.cub.2016.12.018

Linz DM, Hara Y, Deem KD, Kuraku S, Hayashi S, Tomoyasu Y. 2023. Transcriptomic exploration of the coleopteran wings reveals insight into the evolution of novel structures associated with the beetle elytron. J Exp Zool B Mol Dev Evol 340:197–213. https://doi.org/10.1002/jez.b.23188

Linz DM, Hu AW, Sitvarin MI, Tomoyasu Y. 2016. Functional value of elytra under various stresses in the red flour beetle, *Tribolium Castaneum*. Sci Rep 6:34813. https://doi.org/10.1038/srep34813

Lozano-Fernandez J, Giacomelli M, Fleming JF, Chen A, Vinther J, Thomsen PF, Glenner H, Palero F, Legg DA, Iliffe TM et al. 2019. Pancrustacean evolution illuminated by taxon-rich genomic-scale data sets with an expanded remipede sampling. Genome Biol Evol 11:2055–70. https://doi.org/10.1093/gbe/evz097

Lyu C, Abbott LF, Maimon G. 2022. Building an allocentric travelling direction signal via vector computation. Nature 601:92–7. https://doi.org/10.1038/s41586-021-04067-0

Ma KY, Chirarattananon P, Fuller SB, Wood RJ. 2013. Controlled flight of a biologically inspired, insect-scale robot. Science 340:603–7. https://doi.org/10.1126/science.1231806

Marden JH. 2000. Variability in the size, composition, and function of insect flight muscles. Annu Rev Physiol 62:157–78. https://doi.org/10.1146/annurev.physiol.62.1.157

Marden JH. 2013a. Reanalysis and experimental evidence indicate that the earliest trace fossil of a winged insect was a surface-skimming neopteran. Evolution 67:274–80. https://doi.org/10.1111/j.1558-5646.2012.01743.x

Marden JH. 2013b. Nature's inordinate fondness for metabolic enzymes: why metabolic enzyme loci are so frequently targets of selection. Mol Ecol 22:5743–64. https://doi.org/10.1111/mec.12534

Marden JH, Allen LR. 2002. Molecules, muscles, and machines: universal performance characteristics of motors. Proc Natl Acad Sci USA 99:4161–6. https://doi.org/10.1073/pnas.022052899

Marden JH, Fescemyer HW, Schilder RJ, Doerfler WR, Vera JC, Wheat CW. 2013. Genetic variation in hif signaling underlies quantitative variation in physiological and life-history traits within lowland butterfly populations. Evolution 67:1105–15. https://doi.org/10.1111/evo.12004

Marden JH, Kramer MG. 1994. Surface-skimming stoneflies: a possible intermediate stage in insect flight evolution. Science 266:427–30. https://doi.org/10.1126/science.266.5184.427

Marden JH, Kramer MG. 1995. Locomotor performance of insects with rudimentary wings. Nature 377:332–4. https://doi.org/10.1038/377332a0

Marden JH, Langford EA, Robertson MA, Fescemyer HW. 2021. Alleles in metabolic and oxygen-sensing genes are associated with antagonistic pleiotropic effects on life history traits and population fitness in an ecological model insect. Evolution 75:116–29. https://doi.org/10.1111/evo.14095

Marden JH, O'Donnell BC, Thomas MA, Bye JY. 2000. Surface-skimming stoneflies and mayflies: the taxonomic and mechanical diversity of two-dimensional aerodynamic locomotion. Physiol Biochem Zool 73:751–64. https://doi.org/10.1086/318109

Marden JH, Thomas MA. 2003. Rowing locomotion by a stonefly that possesses the ancestral pterygote condition of co-occurring wings and abdominal gills. Biol J Linn Soc 79:341–9. https://doi.org/10.1046/j.1095-8312.2003.00192.x

Martynov AB. 1925. Über zwei grundtypen der flügel bei den insecten und ihre evolution. Zeitschrift Für Morphologie Und Ökologie Der Tiere 4:465–501.

Matheson AMM, Lanz AJ, Medina AM, Licata AM, Currier TA, Syed MH, Nagel KI. 2022. A neural circuit for wind-guided olfactory navigation. Nat Commun 13:4613. https://doi.org/10.1038/s41467-022-32247-7

Mattila ALK. 2015. Thermal biology of flight in a butterfly: genotype, flight metabolism, and environmental conditions. Ecol Evol 5:5539–51. https://doi.org/10.1002/ece3.1758

Medved V, Marden JH, Fescemyer HW, Der JP, Liu J, Mahfooz N, Popadić A. 2015. Origin and diversification of wings: insights from a neopteran insect. Proc Nat Acad Sci 112:15946–51. https://doi.org/10.1073/pnas.1509517112

Melis JM, Siwanowicz I, Dickinson MH. 2024. Machine learning reveals the control mechanics of an insect wing hinge. *bioRxiv*.2023.2006.2029.547116. https://doi.org/10.1101/2023.06.29.547116 (Accessed 7/20/2024)

Menail HA, Cormier SB, Ben Youssef M, Jørgensen LB, Vickruck JL, Morin P, Boudreau LH, Pichaud N. 2022. Flexible thermal sensitivity of mitochondrial oxygen consumption and substrate oxidation in flying insect species. Front Physiol 13:897174. https://doi.org/10.3389/fphys.2022.897174

Menz MHM, Scacco M, Bürki-Spycher H-M, Williams HJ, Reynolds DR, Chapman JW, Wikelski M. 2022. Individual tracking reveals long-distance flight-path control in a nocturnally migrating moth. Science 377:764–8. https://doi.org/10.1126/science.abn1663

Mesquita RD, Gaviraghi A, Gonçalves RLS, Vannier-Santos MA, Mignaco JA, Fontes CFL, Machado LESF, Oliveira MF. 2021. Cytochrome *c* oxidase at full thrust: regulation and biological consequences to flying insects. Cells 10:470. https://doi.org/10.3390/cells10020470

Minter M, Pearson A, Lim KS, Wilson K, Chapman JW, Jones CM. 2018. The tethered flight technique as a tool for studying life-history strategies associated with migration in insects. Ecol Entomol 43:397–411. https://doi.org/10.1111/een.12521

Misof B, Liu S, Meusemann K, Peters RS, Donath A, Mayer C, Frandsen PB, Ware J, Flouri T, Beutel RG et al. 2014. Phylogenomics resolves the timing and pattern of insect evolution. Science 346:763–7. https://doi.org/10.1126/science.1257570

Mitchell R, Shaverdian S, Dacke M, Webb B. 2023. A model of cue integration as vector summation in the insect brain. Proc R Soc B Biol Sci 290:20230767. https://doi.org/10.1098/rspb.2023.0767

Miyan JA, Ewing AW, Lighthill MJ. 1985. How diptera move their wings: a re-examination of the wing base articulation and muscle systems concerned with flight. Philos Trans R Soc B, Biol Sci 311:271–302. https://doi.org/10.1098/rstb.1985.0154

Montooth KL, Marden JH, Clark AG. 2003. Mapping determinants of variation in energy metabolism, respiration and flight in *Drosophila*. Genetics 165:623–35. https://doi.org/10.1093/genetics/165.2.623

Mouritsen H, Frost BJ. 2002. Virtual migration in tethered flying monarch butterflies reveals their orientation mechanisms. Proc Nat Acad Sci 99:10162–6. https://doi.org/10.1073/pnas.152137299

Muijres FT, Elzinga MJ, Iwasaki NA, Dickinson MH. 2015. Body saccades of *Drosophila* consist of stereotyped banked turns. J Exp Biol 218:864–75. https://doi.org/10.1242/jeb.114280

Muijres FT, Iwasaki NA, Elzinga MJ, Melis JM, Dickinson MH. 2017. Flies compensate for unilateral wing damage through modular adjustments of wing and body kinematics. Interface Focus 7:20160103. https://doi.org/10.1098/rsfs.2016.0103

Mussells PP, Zhang L, Parache V, Abbott LF, Maimon G. 2024. Converting an allocentric goal into an egocentric steering signal. Nature 626:808–18. https://doi.org/10.1038/s41586-023-07006-3

Nalbach G. 1989. The gear change mechanism of the blowfly (*Calliphora erythrocephala*) in tethered flight. J Comp Physiol A 165:321–31. https://doi.org/10.1007/BF00619351

Namiki S, Dickinson MH, Wong AM, Korff W, Card GM. 2018. The functional organization of descending sensory-motor pathways in *Drosophila*. eLife 7:e34272. https://doi.org/10.7554/eLife.34272

Namiki S, Ros IG, Morrow C, Rowell WJ, Card GM, Korff W, Dickinson MH. 2022. A population of descending neurons that regulates the flight motor of *Drosophila*. Curr Biol 32:1189–1196.e1186. https://doi.org/10.1016/j.cub.2022.01.008

Naranjo SE. 2019. Assessing insect flight behavior in the laboratory: a primer on flight mill methodology and what can be learned. Ann Entomol Soc Am 112:182–99. https://doi.org/10.1093/aesa/say041

Nesbit RL, Hill JK, Woiwod IP, Sivell D, Bensusan KJ, Chapman JW. 2009. Seasonally adaptive migratory headings mediated by a sun compass in the painted lady butterfly, *Vanessa cardui*. Anim Behav 78:1119–25. https://doi.org/10.1016/j.anbehav.2009.07.039

Nicholson DB, Ross AJ, Mayhew PJ. 2014. Fossil evidence for key innovations in the evolution of insect diversity. Proc R Soc B: Biol Sci 281:20141823. https://doi.org/10.1098/rspb.2014.1823

Nicolson SW, Louw GN. 1982. Simultaneous measurement of evaporative water loss, oxygen consumption, and thoracic temperature during flight in a carpenter bee. J Exp Zool 222:287–96. https://doi.org/10.1002/jez.1402220311

Niitepõld K. 2010. Genotype by temperature interactions in the metabolic rate of the glanville fritillary butterfly. J Exp Biol 213:1042–8. https://doi.org/10.1242/jeb.034132

Niitepõld K. 2019. Effects of flight and food stress on energetics, reproduction, and lifespan in the butterfly *Melitaea cinxia*. Oecologia 191:271–83. https://doi.org/10.1007/s00442-019-04489-8

Niitepõld K, Mattila ALK, Harrison PJ, Hanski I. 2011. Flight metabolic rate has contrasting effects on dispersal in the two sexes of the glanville fritillary butterfly. Oecologia 165:847–54. https://doi.org/10.1007/s00442-010-1886-8

Niitepõld K, Parry HA, Harris NR, Appel AG, De Roode JC, Kavazis AN, Hood WR. 2022. Flying on empty: reduced mitochondrial function and flight capacity in food-deprived monarch butterflies. J Exp Biol 225:jeb244431. https://doi.org/10.1242/jeb.244431

Niitepõld K, Smith AD, Osborne JL, Reynolds DR, Carreck NL, Martin AP, Marden JH, Ovaskainen O, Hanski I. 2009. Flight metabolic rate and pgi genotype influence butterfly dispersal rate in the field. Ecology 90:2223–32. https://doi.org/10.1890/08-1498.1

Nishida K, Adachi H, Moriyama M, Futahashi R, Hanson PE, Kondo S. 2023. Butterfly wing color made of pigmented liquid. Cell Rep 42:112917. https://doi.org/10.1016/j.celrep.2023.112917

Niwa N, Akimoto-Kato A, Niimi T, Tojo K, Machida R, Hayashi S. 2010. Evolutionary origin of the insect wing via integration of two developmental modules. Evol Dev 12:168–76. https://doi.org/10.1111/j.1525-142X.2010.00402.x

Norberg RÅ. 1972. The pterostigma of insect wings an inertial regulator of wing pitch. J Comp Physiol 81:9–22. https://doi.org.10.1007/BF00693547

Nordström K. 2012. Neural specializations for small target detection in insects. Curr Opin Neurobiol 22:272–8. https://doi.org/10.1016/j.conb.2011.12.013

O'carroll D. 1993. Feature-detecting neurons in dragonflies. Nature 362:541–3. https://doi.org/10.1038/362541a0

Ohde T, Mito T, Niimi T. 2022. A hemimetabolous wing development suggests the wing origin from lateral tergum of a wingless ancestor. Nat Commun 13:979. https://doi.org/10.1038/s41467-022-28624-x

Ohde T, Yaginuma T, Niimi T. 2013. Insect morphological diversification through the modification of wing serial homologs. Science 340:495–8. https://doi.org/10.1126/science.1234219

Olberg RM. 1981. Object- and self-movement detectors in the ventral nerve cord of the dragonfly. J Comp Physiol 141:327–34. https://doi.org/10.1007/BF00609935

Orchard I, Ramirez J, Lange A. 1993. A multifunctional role for octopamine in locust flight. Annu Rev Entomol 38:227–49. https://doi.org/10.1146/annurev.en.38.010193.001303

Osborne JL, Clark SJ, Morris RJ, Williams IH, Riley JR, Smith AD, Reynolds DR, Edwards AS. 1999. A landscape-scale study of bumble bee foraging range and constancy, using harmonic radar. J Appl Ecol 36:519–33. https://doi.org/10.1046/j.1365-2664.1999.00428.x

Ovaskainen O, Smith AD, Osborne JL, Reynolds DR, Carreck NL, Martin AP, Niitepõld K, Hanski I. 2008. Tracking butterfly movements with harmonic radar reveals an effect of population age on movement distance. Proc Nat Acad Sci 105:19090–5. https://doi.org/10.1073/pnas.0802066105

Pass G. 1998. Accessory pulsatile organsIn: Microscopic anatomy of invertebrates: 11B: Insecta New York (NY): Wiley-Liss, Inc. p.621–40.

Pass G. 2018. Beyond aerodynamics: the critical roles of the circulatory and tracheal systems in maintaining insect wing functionality. Arthropod Struct Dev 47:391–407. https://doi.org/10.1016/j.asd.2018.05.004

Pekny JE, Smith PB, Marden JH. 2018. Enzyme polymorphism, oxygen and injury: a lipidomic analysis of flight-induced oxidative damage in a succinate dehydrogenase d (sdhd)-polymorphic insect. J Exp Biol 221: jeb171009.

Pennycuick CJ. 2008. Modelling the flying bird. Burlington (MA): Academic Press.

Phan HV, Park HC. 2020. Mimicking nature's flyers: a review of insect-inspired flying robots. Curr Opin Insect Sci 42:70–5. https://doi.org/10.1016/j.cois.2020.09.008

Pons A. 2023. The self-oscillation paradox in the flight motor of *Drosophila melanogaster*. J R Soc Interface 20:20230421. https://doi.org/10.1098/rsif.2023.0421

Pons A, Beatus T. 2022. Distinct Forms of resonant optimality within insect indirect flight motors. J R Soc Interface 19:20220080. https://doi.org/10.1098/rsif.2022.0080

Pons A, Perl I, Ben-Dov O, Maya R, Beatus T. 2023. Solving the thoracic inverse problem in the fruit fly. Bioinspir Biomim 18:046002. https://doi.org/10.1088/1748-3190/accc23

Prange HD. 1996. Evaporative cooling in insects. J Insect Physiol 42:493–9. https://doi.org/10.1016/0022-1910(95)00126-3

Pringle JWS. 1957. Insect flight. New York (NY): Cambridge University Press.

Prokop J, Nel A, Hoch I. 2005. Discovery of the oldest known pterygota in the lower carboniferous of the Upper Silesian basin in the Czech Republic (Insecta: Archaeorthoptera). Geobios 38:383–7. https://doi.org/10.1016/j.geobios.2003.11.006

Prokop J, Pecharová M, Nel A, Hörnschemeyer T, Krzemińska E, Krzemiński W, Engel MS. 2017. Paleozoic nymphal wing pads support dual model of insect wing origins. Curr Biol 27:263–9. https://doi.org/10.1016/j.cub.2016.11.021

Prokop J, Rosová K, Krzemińska E, Krzemiński W, Nel A, Engel MS. 2022. Abdominal serial homologues of wings in paleozoic insects. Curr Biol 32:3414–3422.e3411. https://doi.org/10.1016/j.cub.2022.06.024

Prokop J, Rosová K, Leipner A, Sroka P. 2023. Thoracic and abdominal outgrowths in early pterygotes: a clue to the common ancestor of winged insects? Commun Biol 6:1262. https://doi.org/10.1038/s42003-023-05568-6

Pueyo JI, Galindo MI, Bishop SA, Couso JP. 2000. Proximal-distal leg development in *Drosophila* requires the apterous gene and the lim1 homologue dlim1. Development 127:5391–402. https://doi.org/10.1242/dev.127.24.5391

Rajabi H, Bazargan P, Pourbabaei A, Eshghi S, Darvizeh A, Gorb SN, Taylor D, Dirks JH. 2017. Wing cross veins: an efficient biomechanical strategy to mitigate fatigue failure of insect cuticle. Biomech Model Mechan 16:1947–55. https://doi.org/10.1007/s10237-017-0930-6

Rajabi H, Dirks J-H, Gorb SN. 2020. Insect wing damage: causes, consequences and compensatory mechanisms. J Exp Biol 223:215194. https://doi.org/10.1242/jeb.215194

Ranjan KS, Pawar AA, Roy A, Saha S. 2023. Transoceanic migration network of dragonfly *Pantala Flavescens*: origin, dispersal and timing. Front Ecol Evol 11:1152384. https://doi.org/10.3389/fevo.2023.1152384

Rankin MA, Burchsted JCA. 1992. The cost of migration in insects. Annu Rev Entomol 37:533–59. https://doi.org/10.1146/annurev.en.37.010192.002533

Rasnitsyn AP. 1981. A modified paranotal theory of insect wing origin. J Morphol 168:331–8. https://doi.org/10.1002/jmor.1051680309

Rauhamäki V, Wolfram J, Jokitalo E, Hanski I, Dahlhoff EP. 2014. Differences in the aerobic capacity of flight muscles between butterfly populations and species with dissimilar flight abilities. PLoS One 9:e78069. https://doi.org/10.1371/journal.pone.0078069

Reichert H. 1989. Neural mechanisms underlying axial /appendicular steering reactions in locust flight. Am Zool 29:161–9. https://doi.org.10.1093/icb/29.1.161

Reichert H, Rowell CH. 1985. Integration of nonphaselocked exteroceptive information in the control of rhythmic flight in the locust. J Neurophysiol 53:1201–18. https://doi.org.10.1152/jn.1985.53.5.1201

Roberts SP, Harrison JF. 1999. Mechanisms of thermal stability during flight in the honeybee *Apis mellifera*. J Exp Biol 202:1523–33. https://doi.org/10.1242/jeb.202.11.1523

Robertson RM. 2020. Neural control of flight in locusts. In: Whelan PJ, Sharples SA, editors The neural control of movement: Model systems and tools to study locomotor function. Cambridge (MA): Academic Press. p.75–95. https://doi.org/10.1016/B978-0-12-816477-8.00004-1.

Rodríguez E, Weber J-M, Pagé B, Roubik DW, Suarez RK, Darveau C-A. 2015. Setting the pace of life: membrane composition of flight muscle varies with metabolic rate of hovering orchid bees. Proc R Soc B Biol Sci 282:20142232. https://doi.org/10.1098/rspb.2014.2232

Roff DA. 1986. The evolution of wing dimorphism in insects. Evolution 40:1009–20. https://doi.org/10.1111/j.1558-5646.1986.tb00568.x

Roff DA. 1990. The evolution of flightlessness in insects. Ecol Monogr 60:389–421. https://doi.org/10.2307/1943013

Roff DA, Fairbairn DJ. 1991. Wing dimorphisms and the evolution of migratory polymorphisms among the insecta. Am Zool 31:243–51. https://doi.org/10.1093/icb/31.1.243

Ros IG, Omoto JJ, Dickinson MH. 2024. Descending control and regulation of spontaneous flight turns in *Drosophila*. Curr Biol 34:531–540.e535. https://doi.org/10.1016/j.cub.2023.12.047

Ross A. 2022. Evolution: the origin of insect wings revisited. Curr Biol 32:R851–3. https://doi.org/10.1016/j.cub.2022.06.087

Rothe U, Nachtigall W. 1989. Flight of the honey bee. J Comp Physiol B 158:739–49. https://doi.org/10.1007/BF00693012

Sacktor B. 1961. The role of mitochondria in respiratory metabolism of flight muscle. Annu Rev Entomol 6:103–30. https://doi.org/10.1146/annurev.en.06.010161.000535

Salcedo MK, Jun BH, Socha JJ, Pierce NE, Vlachos PP, Combes SA. 2023. Complex hemolymph circulation patterns in grasshopper wings. Commun Biol 6:313. https://doi.org/10.1038/s42003-023-04651-2

Salcedo MK, Socha JJ. 2020. Circulation in insect wings. Integr Comp Biol 60:1208–20. https://doi.org/10.1093/icb/icaa124

Saleh Ziabari O, Li B, Hardy NB, Brisson JA. 2023. Aphid male wing polymorphisms are transient and have evolved repeatedly. Evolution 77:1056–65. https://doi.org/10.1093/evolut/qpad024

Sato H, Berry C, Peeri Y, Baghoomian E, Casey B, Lavella G, Vandenbrooks J, Harrison J, Maharbiz M. 2009. Remote radio control of insect flight. Front Integr Neurosci 3:24. https://doi.org/10.3389/neuro.07.024.2009

Simon S, Blanke A, Meusemann K. 2018. Reanalyzing the palaeoptera problem—the origin of insect flight remains obscure. Arthropod Struct Dev 47:328–38. https://doi.org/10.1016/j.asd.2018.05.002

Simpson JH. 2024. Descending control of motor sequences in *Drosophila*. Curr Opin Neurobiol 84:102822. https://doi.org/10.1016/j.conb.2023.102822

Siviter H, Fisher A, Baer B, Brown MJF, Camargo IF, Cole J, Le Conte Y, Dorin B, Evans JD, Farina W et al. 2023. Protecting pollinators and our food supply: understanding and managing threats to pollinator health. Insectes Soc 70:5–16. https://doi.org/10.1007/s00040-022-00897-x

Smýkal V, Pivarči M, Provazník J, Bazalová O, Jedlička P, Lukšan O, Horák A, Vaněčková H, Beneš V, Fiala I et al. 2020. Complex evolution of insect insulin receptors and homologous decoy receptors, and functional significance of their multiplicity. Mol Biol Evol 37:1775–89. https://doi.org/10.1093/molbev/msaa048

Snodgrass RE. 1935. Principles of insect morphology. New York (NY): McGraw Hill Book Company.

Sotavalta O. 1952. Flight-tone and wing-stroke frequency of insects and the dynamics of insect flight. Nature 170:1057–8. https://doi.org/10.1038/1701057a0

Stevenson PA, Kutsch W. 1987. A reconsideration of the central pattern generator concept for locust flight. J Comp Physiol A 161:115–29. https://doi.org/10.1007/BF00609460

Stone T, Webb B, Adden A, Weddig NB, Honkanen A, Templin R, Wcislo W, Scimeca L, Warrant E, Heinze S. 2017. An anatomically constrained model for path integration in the bee brain. Curr Biol 27:3069–3085.e3011. https://doi.org/10.1016/j.cub.2017.08.052

Strausfeld NJ. 2012. Arthropod brains: evolution, functional elegance, and historical significance. Cambridge (MA): Harvard University Press.

Stupski SD, Breugel FV. 2023. Wind gates search states in free flight. *bioRxiv*.2023.2011.2030.569086. https://doi.org/10.1101/2023.11.30.569086

Suarez RK. 2000. Energy metabolism during insect flight: biochemical design and physiological performance. Physiol Biochem Zool 73:765–71. https://doi.org/10.1086/318112

Sugimoto M, Ogawa N, Yoshizawa K. 2018. Morphology of the elytral base sclerites. Arthropod Struct Dev 47:423–9. https://doi.org/10.1016/j.asd.2018.02.004

Sun B-J, Huebner C, Treidel LA, Clark RM, Roberts KT, Kenagy GJ, Williams CM. 2020. Nocturnal dispersal flight of crickets: behavioural and physiological responses to cool environmental temperatures. Funct Ecol 34:1907–20. https://doi.org/10.1111/1365-2435.13615

Sun J, Ling M, Wu W, Bhushan B, Tong J. 2014. The hydraulic mechanism of the unfolding of hind wings in *Dorcus titanus platymelus* (Order: Coleoptera). Int J Mol Sci 15:6009–18. https://doi.org/10.3390/ijms15046009

Suver MP, Huda A, Iwasaki N, Safarik S, Dickinson MH. 2016. An array of descending visual interneurons encoding self-motion in *Drosophila*. J Neurosci 36:11768–80. https://doi.org/10.1523/jneurosci.2277-16.2016

Talal S, Parmar S, Osgood GM, Harrison JF, Cease AJ. 2023. High carbohydrate consumption increases lipid storage and promotes migratory flight in locusts. J Exp Biol 226:jeb245351. https://doi.org/10.1242/jeb.245351

Tercel MPTG, Veronesi F, Pope TW. 2018. Phylogenetic clustering of wingbeat frequency and flight-associated morphometrics across insect orders. Physiol Entomol 43:149–57. https://doi.org/10.1111/phen.12240

Teulier L, Weber J-M, Crevier J, Darveau C-A. 2016. Proline as a fuel for insect flight: enhancing carbohydrate oxidation in hymenopterans. Proc R Soc B Biol Sci 283:20160333. https://doi.org/10.1098/rspb.2016.0333

Tihelka E, Cai C, Giacomelli M, Lozano-Fernandez J, Rota-Stabelli O, Huang D, Engel MS, Donoghue PCJ, Pisani D. 2021. The evolution of insect biodiversity. Curr Biol 31:R1299–311. https://doi.org/10.1016/j.cub.2021.08.057

Trimarchi JR, Schneiderman AM. 1995. Different neural pathways coordinate *Drosophila* flight initiations evoked by visual and olfactory stimuli. J Exp Biol 198:1099–104. https://doi.org/10.1242/jeb.198.5.1099

Tripathi BK, Irvine KD. 2022. The wing imaginal disc. Genetics 220:iyac020. https://doi.org/10.1093/genetics/iyac020

Trueman JWH, Pfeil BE, Kelchner SA, Yeates DK. 2004. Did stick insects really regain their wings? Syst Entomol 29:138–9. https://doi.org/10.1111/j.0307-6970.2004.00251.x

Tsai C-C, Childers RA, Nan Shi N, Ren C, Pelaez JN, Bernard GD, Pierce NE, Yu N. 2020. Physical and behavioral adaptations to prevent overheating of the living wings of butterflies. Nat Commun 11:551. https://doi.org/10.1038/s41467-020-14408-8

Tucker VA. 1975. The energetic cost of moving about: walking and running are extremely inefficient forms of locomotion. much greater efficiency is achieved by birds, fish—and bicyclists. Am Sci 63:413–9.

Turner-Evans DB, Jayaraman V. 2016. The insect central complex. Curr Biol 26:R453–7. https://doi.org/10.1016/j.cub.2016.04.006

Van Breugel F, Jewell R, Houle J. 2022. Active anemosensing hypothesis: how flying insects could estimate ambient wind direction through sensory integration and active movement. J R Soc Interface 19:20220258. https://doi.org/10.1098/rsif.2022.0258

Vellichirammal NN, Gupta P, Hall TA, Brisson JA. 2017. Ecdysone signaling underlies the pea aphid transgenerational wing polyphenism. Proc Nat Acad Sci 114:1419–23. https://doi.org/10.1073/pnas.1617640114

Venken KJT, Simpson JH, Bellen HJ. 2011. Genetic manipulation of genes and cells in the nervous system of the fruit fly. Neuron 72:202–30. https://doi.org/10.1016/j.neuron.2011.09.021

Vickers NJ, Christensen TA, Hildebrand JG. 1998. Integrating behavior with neurobiology: odor-mediated moth flight and olfactory discrimination by glomerular arrays. Integr Biol: Issues, News, Rev 1:224–30. https://doi.org/10.1002/(SICI)1520-6602(1998)1:6⟨224::AID-INBI4⟩3.0.CO;2-Q

Von Reyn CR, Breads P, Peek MY, Zheng GZ, Williamson WR, Yee AL, Leonardo A, Card GM. 2014. A spike-timing mechanism for action selection. Nat Neurosci 17:962–70. https://doi.org/10.1038/nn.3741

Von Reyn CR, Nern A, Williamson WR, Breads P, Wu M, Namiki S, Card GM. 2017. Feature integration drives probabilistic behavior in the *Drosophila* escape response. Neuron 94:1190–1204.e1196. https://doi.org/10.1016/j.neuron.2017.05.036

Walker SM, Schwyn DA, Mokso R, Wicklein M, Müller T, Doube M, Stampanoni M, Krapp HG, Taylor GK. 2014. In vivo time-resolved microtomography reveals the mechanics of the blowfly flight motor. PLoS Biol 12:e1001823. https://doi.org/10.1371/journal.pbio.1001823

Walker SM, Thomas ALR, Taylor GK. 2012. Operation of the alula as an indicator of gear change in hoverflies. J R Soc Interface 9:1194–207. https://doi.org/10.1098/rsif.2011.0617

Wang Y, Yin Y, Zheng G, Yao H. 2020. Driving mechanism of dragonfly’s wing flapping pattern for liquid circulation inside wing. Anim Biol 71:85–101. https://doi.org/10.1163/15707563-bja10048

Waters JM, Emerson BC, Arribas P, Mcculloch GA. 2020. Dispersal reduction: causes, genomic mechanisms, and evolutionary consequences. Trends Ecol Evol 35:512–22. https://doi.org/10.1016/j.tree.2020.01.012

Watt WB. 1968. adaptive significance of pigment polymorphisms in *Colias* Butterflies. I. variation of melanin pigment in relation to thermoregulation. Evolution 22:437–58. https://doi.org/10.2307/2406873

Watt WB, Wheat CW, Meyer EH, Martin J-F. 2003. Adaptation at specific Loci. Vii. natural selection, dispersal and the diversity of molecular–functional variation patterns among butterfly species complexes (Colias: Lepidoptera, Pieridae). Mol Ecol 12:1265–75. https://doi.org/10.1046/j.1365-294X.2003.01804.x

Weis-Fogh T. 1960. A rubber-like protein in insect cuticle. J Exp Biol 37:889–907. https://doi.org/10.1242/jeb.37.4.889

Weis-Fogh T. 1973. Quick estimates of flight fitness in hovering animals, including novel mechanisms for lift production. J Exp Biol 59:169–230. https://doi.org/10.1242/jeb.59.1.169

Weis-Fogh T, Uvarov BP. 1952. Fat combustion and metabolic rate of flying locusts (*Schistocerca gregaria*). Philos Trans R Soc B, Biol Sci 237:1–36. https://doi.org/10.1098/rstb.1952.0011

Westeinde EA, Kellogg E, Dawson PM, Lu J, Hamburg L, Midler B, Druckmann S, Wilson RI. 2024. Transforming a head direction signal into a goal-oriented steering command. Nature 626:819–26. https://doi.org/10.1038/s41586-024-07039-2

Wheat CW, Watt WB, Pollock DD, Schulte PM. 2005. From DNA to fitness differences: sequences and structures of adaptive variants of *Colias* phosphoglucose isomerase (pgi). Mol Biol Evol 23:499–512. https://doi.org/10.1093/molbev/msj062

Whiting MF, Bradler S, Maxwell T. 2003. Loss and recovery of wings in stick insects. Nature 421:264–7. https://doi.org/10.1038/nature01313

Wilmsen SM, Dzialowski E. 2023. Substrate use and temperature effects in flight muscle mitochondria from an endothermic insect, the hawkmoth *Manduca Sexta*. Comp Biochem Physiol A: Mol Integr Physiol 281:111439. https://doi.org/10.1016/j.cbpa.2023.111439

Wilson DM. 1961. The central nervous control of flight in a locust. J Exp Biol 38:471–90. https://doi.org/10.1242/jeb.38.2.471

Wipfler B, Letsch H, Frandsen PB, Kapli P, Mayer C, Bartel D, Buckley TR, Donath A, Edgerly-Rooks JS, Fujita M et al. 2019. Evolutionary history of polyneoptera and its implications for our understanding of early winged insects. Proc Nat Acad Sci 116:3024–9. https://doi.org/10.1073/pnas.1817794116

Wisser A. 1988. Wing beat of *Calliphora erythrocephala*: turning axis and gearbox of the wing base (insecta, diptera). Zoomorphology 107:359–69. https://doi.org/10.1007/BF00312219

Wolf TJ, Schmid-Hempel P, Ellington CP, Stevenson RD. 1989. Physiological correlates of foraging efforts in honey-bees: oxygen consumption and nectar load. Funct Ecol 3:417–24. https://doi.org/10.2307/2389615

Xu H-J, Xue J, Lu B, Zhang X-C, Zhuo J-C, He S-F, Ma X-F, Jiang Y-Q, Fan H-W, Xu J-Y et al. 2015. Two insulin receptors determine alternative wing morphs in planthoppers. Nature 519:464–7. https://doi.org/10.1038/nature14286

Xu H-J, Zhang C-X. 2017. Insulin receptors and wing dimorphism in rice planthoppers. Philos Trans R Soc B Biol Sci 372:20150489. https://doi.org/10.1098/rstb.2015.0489

Yanoviak SP, Dudley R, Kaspari M. 2005. Directed aerial descent in canopy ants. Nature 433:624–6. https://doi.org/10.1038/nature03254

Yanoviak SP, Kaspari M, Dudley R. 2009. Gliding hexapods and the origins of insect aerial behaviour. Biol Lett 5:510–2. https://doi.org10.1098/rsbl.2009.0029

Zera AJ. 2016. Juvenile hormone and the endocrine regulation of wing polymorphism in insects: new insights from circadian and functional-genomic studies in *Gryllus* crickets. Physiol Entomol 41:313–26. https://doi.org/10.1111/phen.12166

Zhan S, Zhang W, Niitepõld K, Hsu J, Haeger JF, Zalucki MP, Altizer S, De Roode JC, Reppert SM, Kronforst MR. 2014. The genetics of monarch butterfly migration and warning colouration. Nature 514:317–21. https://doi.org/10.1038/nature13812