Corrugation, flexibility and wear of fly wings

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List of abbreviations

AIFIT aerodynamics of insect flight in turbulent flows
CFD computational fluid dynamics
DLM dorsal longitudinal muscles
DVM dorso-ventral muscles
LEV leading edge vortex

List of variables

Variable	Dimension	Description
A_{char}	L^2	characteristic area
c	L	wing chord
c_{D}	dimensionless	drag coefficient
c_{L}	dimensionless	lift coefficient
d_{char}	L	characteristic length
E	$\mathrm{M\,L^{-1}\,T^{-2}}$	Young's modulus of elasticity
EI	$\mathrm{M\,L^3\,T^{-2}}$	flexural stiffness
F_{D}	$\mathrm{M\,L\,T^{-2}}$	drag force
F_{L}	$\mathrm{M\,L\,T^{-2}}$	lift force
f	$\mathrm{T^{-1}}$	wingbeat frequency
I	L^4	second moment of area, area moment of inertia
J	dimensionless	advance ratio
k	$\mathrm{M\,T^{-2}}$	spring stiffness
M	dimensionless	Mach number
$n_{\text{measurement}}$	dimensionless	number of available data points
R	L	wing length
r	L	position along wing axis
Re	dimensionless	Reynolds number
S_k	L^{k+2}	k-th moment of wing area
$\vec{U}_\infty$	$\mathrm{L\,T^{-1}}$	free-stream fluid velocity relative to object, true air speed
u_{char}	$\mathrm{L\,T^{-1}}$	characteristic speed
$\bar{u}_{\text{tip}}$	$\mathrm{L\,T^{-1}}$	average wing tip speed
u_{wing}	$\mathrm{L\,T^{-1}}$	wing speed relative to body
$\mathit{Æ}$	dimensionless	aspect ratio
α	angle	(geometric) angle of attack
α_{eff}	angle	effective angle of attack
α_{feather}	angle	feathering angle
η	angle	stroke plane angle
θ	angle	deviation angle, elevation angle

μ	$M L^{-1} T^{-1}$	dynamic viscosity
ν	$L^2 T^{-1}$	kinematic viscosity
ρ	$M L^{-3}$	density
Φ	angle	stroke amplitude
ϕ	angle	positional angle, stroke angle

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Chapter 1

Bilingual summary of thesis

1.1 Abstract

Insects were the first animals to evolve active flight and today several million species are thought to exist in multiple habitats. The aerodynamic principles of insect flight are largely understood, but more detailed investigations require species-specific data. Although knowledge on the properties of insect wings is key in flight research, detailed studies on wings of intact and living animals are rare. Corrugation due to wing veins determines the wings' three-dimensional structure, thereby influencing wing stiffness and thus deformation behaviour during wing flapping. The significance of corrugation for flight aerodynamics is the subject of an ongoing debate. Thus, this thesis investigated the mechanical structures and properties of wings in three dipteran species with different body sizes: *Drosophila melanogaster*, *Musca domestica* and *Calliphora vomitoria*. Fluid dynamic simulations of wing models based on micro-computed tomography scans indicate that wing venation is aligned with areas of strong dorso-ventral pressure differences occurring during flight. In contrast to studies on dragonfly wings at high Reynolds numbers, wing corrugation in flies has a negligible effect on aerodynamic performance, and no trapped vortices exist in the corrugation valleys. These observations support the assumption that corrugation structurally strengthens the wing without improving its aerodynamic performance. To quantify wing stiffness in living flies, wings were point loaded and deformation calculated via symmetry-based and curvature-based approaches. Data show that the stiffness between wing root and a point near the wing's aerodynamic centre is $0.6\,\mathrm{N\,m^{-1}}$ in *D. melanogaster*, $0.7\,\mathrm{N\,m^{-1}}$ in *M. domestica* and $2.6\,\mathrm{N\,m^{-1}}$ in *C. vomitoria*. In *M. domestica*, wings appear more flexible when forces are applied to the dorsal compared to the ventral side. Areas at the wing tip and trailing edge are more flexible than the strongly-veined areas near the leading edge. The haemolymph inside veins keeps the wing cuticle pliant. A loss of moisture in veins of older animals and animals with damaged wings is thought to increase wing stiffness, changing aerodynamic performance and making wings susceptible to further damage. Recordings of wing damage progression in *M. domestica* show that area loss follows a characteristic progression, although its timing varies between sexes and individuals. Wing damage becomes visible after approximately 6 h of locomotor activity. Flight can no longer be maintained at an area loss of 10 % to 34 % and a left-right wing area asymmetry of approximately 25 %. The above results were published as three peer-reviewed articles. In conclusion, the data presented in this thesis help to understand mechanisms and limits of flight force production in differently-sized insects during wing flapping and thus constitute a valuable basis for further research on the ecology and biophysics of insect flight.

1.2 Zusammenfassung

Insekten waren die ersten Tiere, die aktiven Flug entwickelten, und heute geht man von der Existenz mehrerer Millionen Arten in verschiedenen Lebensräumen aus. Die aerodynamischen Prinzipien des Insektenflugs sind weitgehend verstanden, aber für detailliertere Untersuchungen sind artspezifische Daten notwendig. Obwohl Wissen über die Eigenschaften von Insektenflügeln wichtig für die Flugforschung sind, sind detaillierte Studien zu Flügeln intakter und lebendiger Tiere selten. Fältelung aufgrund von Flügeladern bestimmt die dreidimensionale Flügelstruktur, welche die Steifigkeit und daher das Verformungsverhalten während des Schlagens des Flügels beeinflusst. Die Bedeutung von Fältelung für Flugaerodynamik wird aktiv diskutiert. Daher hat die vorliegende Arbeit die mechanischen Strukturen und Eigenschaften von Flügeln dreier Dipterenarten verschiedener Körpergröße untersucht: *Drosophila melanogaster*, *Musca domestica* und *Calliphora vomitoria*. Fluiddynamische Simulationen mikrocomputertomographiebasierter Flügelmodelle deuten darauf hin, dass die Flügeläderung mit Bereichen starker dorso-ventraler Druckunterschiede im Flug zusammenfällt. Im Gegensatz zu Studien an Libellenflügeln bei hohen Reynoldszahlen hat die Flügelfältelung bei Fliegen einen vernachlässigbaren Effekt auf die aerodynamische Leistung und in den Tälern der Falten existieren keine gefangenen Wirbel. Diese Beobachtungen stützen die Annahme, dass die Fältelung den Flügel strukturell verstärkt ohne seine aerodynamischen Eigenschaften zu verbessern. Um Flügelsteifigkeit in lebenden Fliegen zu quantifizieren, wurden Flügel punktbelastet und die Verformung über symmetrie- und krümmungsbasierte Ansätze errechnet. Die Daten zeigen, dass die Steifigkeit zwischen Flügelwurzel und einer Stelle nahe dem aerodynamischen Zentrum des Flügels bei *D. melanogaster* $0.6\,\mathrm{N\,m^{-1}}$ ist, bei *M. domestica* $0.7\,\mathrm{N\,m^{-1}}$ und bei *C. vomitoria* $2.6\,\mathrm{N\,m^{-1}}$. Flügel von *M. domestica* scheinen bei dorsaler Krafteinwirkung flexibler zu sein als bei ventraler. Bereiche an Flügelspitze und -hinterkante sind flexibler als die stark geäderten Bereiche nahe der Vorderkante. Die Hämolymphe in den Adern hält die Flügelkutikula biegsam. Es wird angenommen, dass Feuchtigkeitsverlust in den Adern älter Tiere oder Tieren mit beschädigten Flügeln die Flügelsteifigkeit erhöht, was die Aerodynamik verändert und die Flügel anfälliger für weitere Schäden macht. Die Aufzeichnung fortschreitender Flügelschäden bei *M. domestica* zeigt, dass der Flächenverlust einem charakteristischen Muster folgt, auch wenn der zeitliche Ablauf zwischen Geschlechtern und Individuen variiert. Nach etwa 6 h Bewegungsaktivität wird die Flügelabnutzung deutlich. Die Flugfähigkeit ist bei einem Flächenverlust von mehr als 10 % bis 34 % und einer Links-Rechts-Asymmetrie der Flügelfläche von etwa 25 % nicht mehr gegeben. Die obigen Ergebnisse wurden als drei begutachtete Artikel veröffentlicht. Schließlich helfen die in dieser Arbeit vorgestellten Daten, Mechanismen und Grenzen von Flugkrafterzeugung in verschieden großen Insekten beim Flügelschlag zu verstehen. Sie stellen daher eine nützliche Grundlage für weitere Forschung im Bereich der Ökologie und Biophysik des Insektenflugs dar.

Chapter 2

General introduction

2.1 Background

Insects are a very successful group of animals, with recent estimates of around five to six million insect species in total (Stork, 2018). One reason that contributed to this success may be the ability of the Pterygota (winged insects) to fly (Templin, 2000; Mayhew, 2007). This mode of transportation has a lower cost of transport than walking (Schmidt-Nielsen, 1972; Tucker, 1975), and allows wider dispersal (Mayhew, 2007). Flight is assumed to have first appeared in the late Devonian or early Carboniferous, around 300 to 400 million years ago (Alexander and Brown Jr., 1963; Wagner and Liebherr, 1992; Dudley, 2000; Garrouste *et al.*, 2012), making insects the first animals to evolve active flight in a world with tall vegetation and earth-bound predators (Dudley, 2000). Today, there are only few insect species belonging to the Apterygota (non-winged insects; Alexander and Brown Jr., 1963), although secondary loss of wings and/or flight ability is known also within the Pterygota (Wagner and Liebherr, 1992).

Windy habitats appear to favour this secondary loss of flight (e.g. Darwin, 1999, ch. V; Wagner and Liebherr, 1992; Leihy and Chown, 2020). In less extreme habitats, however, insect flight is robust enough to withstand disturbances, even if there is some impact on flight performance (e.g. Chang *et al.*, 2016; Matthews and Sponberg, 2018). The aim of project AIFIT[1] (Aerodynamics of Insect Flight In Turbulent flows) was to develop numerical models of insect wings and their interaction with the surrounding air in order to investigate control strategies used by insects and possible (dis)advantages of properties of the wings. To this end, the project was a cooperation between computational fluid dynamicists and biologists. The project presented here was part of AIFIT and focused on several properties of real insect wings as a basis for understanding some of the intricacies of insect flight.

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Due to the importance of wings for flight, there is selective pressure on their aerodynamic and mechanical properties. There are, however, further constraints and functions wings need to obey and fulfil: wings are used for communication, courtship, mimicry and crypsis, protection, and they carry sensory organs and nerves and possess a circulatory and tracheal system (Wagner and Liebherr, 1992; Dickinson *et al.*, 1997; Pass *et al.*, 2015; Ray *et al.*, 2016; Breitkreuz *et al.*, 2017; Salcedo and Socha, 2020). They need to be grown and expanded to full size (Salcedo and Socha, 2020), and, in groups like beetles, they also are repeatedly unfolded and refolded (Sun *et al.*, 2016a,b). Additionally, wings must be sufficiently robust or otherwise protected from being damaged. For example, the capability of laying them flat against the body (as found in the Neoptera) gives a wider range of motion and might enable the animals to move into small openings without damaging their wings (Martynov, 1925). Therefore, not all properties of a given insect wing are necessarily optimized (only) for flight. They may influence multiple aspects of the wing's function, making trade-offs necessary. Some properties may also have both direct and indirect influence on the wing's aerodynamic properties. For this reason, detailed investigations of insect flight should include the different wing properties and their impact on flight. The project presented here was concerned with investigating the three-dimensional structure and stiffness of fly wings as well as wing damage accumulation.

The wings of insects are corrugated due to their veins, which carry nerves and trachea (Pass, 2018). On the one hand, this corrugation influences the wings' mechanical properties and in turn their deformation during flight (Rees, 1975b). On the other hand, the corrugation itself may affect wing aerodynamics. This can be investigated using physical (e.g. Okamoto *et al.*, 1996; Kesel, 2000) or computational (e.g. Le *et al.*, 2013; Shahzad *et al.*, 2017) models, and was the focus of the first part of the work presented here (Engels *et al.*, 2020).

Depending on wing kinematics, wing flexibility may improve or impair aerodynamic properties (e.g. Nakata and Liu, 2012; Tobing *et al.*, 2017; Fu *et al.*, 2018) and flight stability (Mistick *et al.*, 2016). To investigate its significance, a grasp on wing flexibility as it occurs in nature is necessary. Overall stiffness of wings varies between species, and correlates with wing size (Combes and Daniel, 2003a). Different wing areas have different flexibility, depending on the pattern of veins and wing membrane stiffness (Wootton, 1981). Measuring wing stiffness of specific species therefore provides the basis for further investigations into its (aerodynamic) function. This is the focus of the second part of the project (Wehmann *et al.*, 2019).

Apart from its influence on flight, flexibility might prevent damage to the wings (Mountcastle and Combes, 2014). Over time, insects accumulate wing damage because wings of adult insects do not regenerate (Kříženecký, 1914; Rajabi *et al.*, 2020). Pronounced wing damage is detrimental to flight ability (Ragland and Sohal, 1973; Haas and Cartar, 2008). Wing damage accumulation and longevity both depend on external conditions (e.g. Ragland and Sohal, 1973; Lee *et al.*, 2006), but there is not necessarily a significant correlation between longevity and the

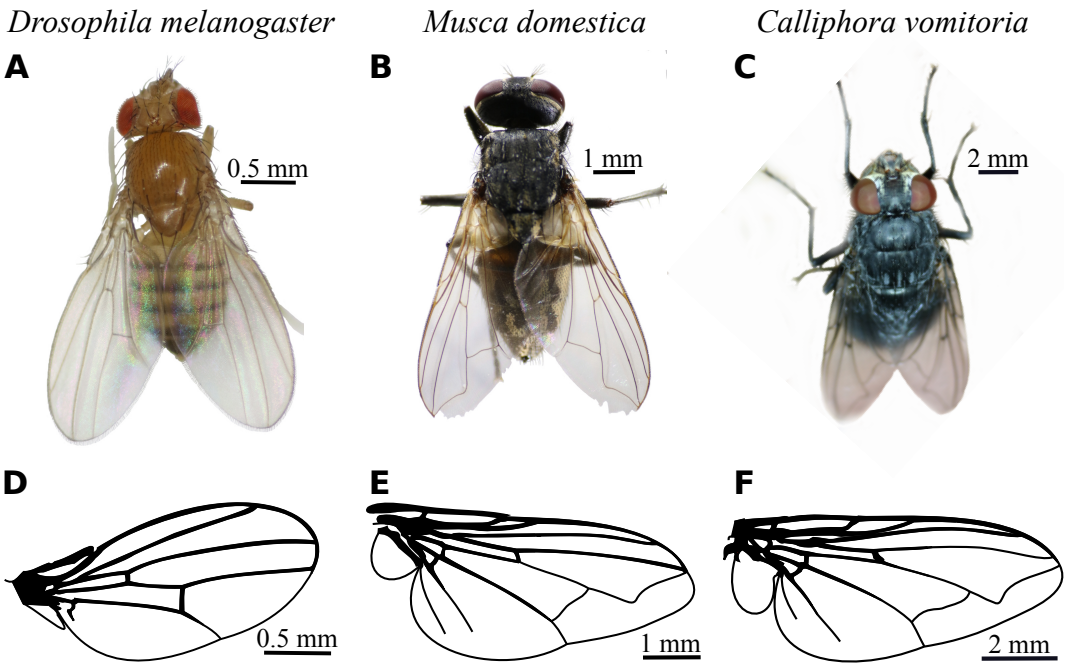

Figure 2.1: The three species used in this thesis: *Drosophila melanogaster* (left), *Musca domestica* (middle), and *Calliphora vomitoria* (right). Upper row (A-C): Females of each species. A: Dorsal view of *D. melanogaster*. B: Dorsal view of *M. domestica* with slight wing damage. C: Dorsal/frontal perspective view of *C. vomitoria*. Lower row (D-F): Schematic representations of corresponding wings.

duration of the maturity period (Novoseltsev *et al.*, 2005). Only while fecundity is still high could wing damage potentially influence fitness, as suggested by Hargrove (1975) or Vance and Roberts (2014). Therefore, studies linking age, activity, wing damage and flight performance are important to understand this particular aspect of insect wings as it relates to the animals' biology. The last part of the project presented here was focused on this (Wehmann *et al.*, 2022).

The present work used flies as experimental animals for several reasons. Diptera (true flies) are good fliers and species vary in size from less than one millimetre to several centimetres (Dettner and Peters, 2010), which allows investigation of size effects within the same order. In contrast to other orders, Diptera have only one pair of wings to generate aerodynamic forces. Their hindwings have evolved into halteres, gyroscopic organs playing a role in flight control (for an overview, see Yarger and Fox, 2016; Dickerson, 2020). This allows research on aerodynamics to focus on only two wings, while investigating species that are also of general interest as model organisms or pest animals. The three fly species used in this project are, in increasing size (see figure 2.1), the vinegar fly *Drosophila melanogaster* Meigen (around 2 mm to 2.5 mm wing length, body mass $1.6\,\text{mg} \pm 0.8\,\text{mg}$, $n = 9$ females), the housefly *Musca domestica* L. ($\sim 7\,\text{mm}$ wing length, body mass $16.4\,\text{mg} \pm 2.9\,\text{mg}$, $n = 4$ females), and the blowfly *Calliphora vomitoria* L. ($\sim 1\,\text{cm}$ wing length, body mass $59.3\,\text{mg} \pm 6.3\,\text{mg}$, $n = 9$ females). Wing corrugation and flexibility were investigated for all three species, while for the wing damage experiments the medium sized *M. domestica* was used.

In the following, some background on the evolution of insect flight and wings will be presented. The structure of the wings will be described and there will be an introduction to the basic principles of insect flight. This lays the groundwork for the discussion of the work done on wing corrugation and its influence on wing aerodynamics, on wing flexibility, and wing damage.

2.2 Insect evolution

To date, at least approximately one million insect species have been described (Engel, 2015; Stork, 2018). This corresponds to more than half of all described species (Mayhew, 2007). Nearly all recent insects belong to the Pterygota (winged insects, Breitkreuz *et al.*, 2017). While secondary loss of wings or flight ability is known in almost all groups of Pterygota (Wagner and Liebherr, 1992), the total number of species known to exhibit total or partial wing loss is small (on the order of hundreds within dipterans, Dettner and Peters, 2010). This is one of the reasons why the ability to fly has been put forward as an explanation for the vast number of insect species we observe today (e.g. Templin, 2000; Mayhew, 2007), although the reasons for the evolutionary success of insects are manifold (Wagner and Liebherr, 1992; Pritchard *et al.*, 1993; Dudley, 2000; Mayhew, 2007; Garrouste *et al.*, 2012; Engel, 2015; Rehm *et al.*, 2011).

2.2.1 Flight as a mode of locomotion

While the contribution of flight ability to the diversification of insects seems logical, the origin of wings was not accompanied by an increased speciation rate, and the existing diversity of arthropods may be best explained by other factors (Mayhew, 2018). Dudley *et al.* (2007) argue that if active flapping flight was a strong evolutionary advantage it seems unlikely that it should only occur in four lineages (pterygote insects, pterosaurs, birds, bats). They do, however, acknowledge that more or less controlled gliding or falling is a rather widespread behaviour.

Indeed, flight has disadvantages as well as advantages as a mode of locomotion. The entire weight of a flier must be supported at all times in air, a medium of low density. Compared to other modes of locomotion, higher metabolic rates are therefore a prerequisite for flight, and it can be costly in terms of increased mortality and reduced fecundity (as discussed in Schmidt-Nielsen, 1972; Roff, 1990; Dudley, 2000). Air resistance, however, is small compared to that of water (Schmidt-Nielsen, 1972), which makes moving through air easier than through water. The cost of transport for flying also tends to be lower than that for walking (Schmidt-Nielsen, 1972; Tucker, 1975). Additional benefits from flight may be the ability to cross rough terrain, travel long distances in shorter times, access new ecological niches, or a way to escape predators (discussed by Alexander and Brown Jr., 1963; Tucker, 1975; Roff, 1990; Dudley, 2000).

In general, moving is costly and the efficiency of locomotor systems therefore of interest. In terms of converting chemical to mechanical energy, insect flight muscles are not very efficient

compared to other muscles. They reach efficiencies of few percent up to approximately 17 % (Ellington, 1985; Josephson, 1985; Josephson and Stevenson, 1991; Josephson *et al.*, 2001; Lehmann, 2001; Smith *et al.*, 2005), while vertebrate muscle efficiencies range from 15 % to 50 % (Lehmann, 2001; Smith *et al.*, 2005). If active insect flight spread in the early Carboniferous as suspected, it fell into a period of time where the atmospheric oxygen concentration was very high (between 350 and 250 million years ago, up to 35 % oxygen). Dudley (2000) discusses that this environment may have mitigated the impact of inefficient muscles, due to more available oxygen and increased lift production because of slightly higher air density. This assumes that oxygen delivery to the muscle was the limiting factor, which may not have been the case (for a critical discussion in the context of gigantism see Butterfield, 2009). Converting the mechanical power of the muscles into aerodynamic power is possible with higher efficiencies, between 26 % (fruit fly) and 55 % (bumblebee with perfect elastic energy storage) for insects, compared with 52 % for a hummingbird (Lehmann, 2001). This results in a total efficiency for the conversion of chemical to aerodynamic energy of only few percent (Lehmann, 2001). The power necessary to produce aerodynamic forces (see section 2.4) is called "aerodynamic power" and is often given per muscle mass of the animal (Ellington, 1985). It is not equivalent to the total power the animal needs to expend because it does not include, for example, the "inertial power" needed for accelerating and decelerating the wing (Ellington, 1985).

2.2.2 Evolution of insect wings

Theories for the evolution of insect wings have to explain how any interim state between "no wing" and "fully winged and capable of flight" created a selective advantage (Alexander and Brown Jr., 1963). Knowledge on the origin of wings may also help explain certain limitations of these organs. Over the last 200 years, two prominent ideas for the evolution of insect wings have been proposed, each in different iterations: the "gill", "exite" or "pleural origin" theory, and the "paranotal" or "tergal origin" theory. There have also been other ideas, like wings originally being used for thermoregulation (Kingsolver and Koehl, 1985) or evolving from parapodia (see Alexander and Brown Jr., 1963; Wigglesworth, 1963, for explanations and discussions of this hypothesis). The basic concepts of the two most prominent theories will be explained here.

Pleural origin. The exite origin theory, originally put forward in the 19th century, posits that wings are homologous to nymphal gills (summarized in Alexander and Brown Jr., 1963), gill plates or styli (Wigglesworth, 1973). Articulated wings may have evolved from structures carrying gills (Wigglesworth, 1973) or leg exites (explained in Dickinson *et al.*, 1997), or be homologous to crustacean epipodites (Averof and Cohen, 1997, but see Jockusch and Nagy, 1997; Boxshall, 2004, with the last proposing co-option of the same genes). While the gill theory explains the articulation of wings and their usefulness before flight, the actual transition from water to air is not clear (Alexander and Brown Jr., 1963).

Tergal origin. The paranotal origin theory suggests that insect wings developed from thoracic structures. This theory is explained in detail by Alexander and Brown Jr. (1963), Wigglesworth (1963) and Kukalova-Peck (1978). A flattening of the notum may have provided protection against predators, better leaf mimicry, and easier access to small cracks (summarized in Alexander and Brown Jr., 1963). The lateral extensions of the notum may then have helped bigger insects to glide when jumping or falling off vegetation (see Flower, 1964; Wigglesworth, 1963; Pass *et al.*, 2015, for more detailed discussions). In contrast to the pleural origin theory, the tergal origin theory cannot readily explain wing articulation. It does, however, more easily explain the origin of flight via postural control during falls and jumps.

Dual origin. A currently widely accepted answer to the question of whether the exite or the paranotal theory is valid is that both are true. This has been mentioned as early as Crampton (1916), who asked whether wings were "tergal, pleural (or both) in nature", but the idea has only gained attention in the last decade via genetic approaches (see for example Niwa *et al.*, 2010; Clark-Hachtel *et al.*, 2013; Clark-Hachtel and Tomoyasu, 2016; Linz and Tomoyasu, 2018; Medved *et al.*, 2015; Hu *et al.*, 2019; Requena *et al.*, 2017; Prokop *et al.*, 2017). While the details are still not clear, a growing body of evidence has bolstered the idea of a dual origin of wings, reconciling two centuries of arguments for and against pleural and tergal wing origins. These ideas about the origin of wings are also consistent with the fact that there are no muscles inside the wings, which instead rely on muscles at their base and their mechanical properties to achieve advantageous conformations during flight (Wootton, 1999; Rubio *et al.*, 2018).

Whether the wings' precursors were useful for breathing, protection, or other functions, one important function of most insect wings today is undoubtedly flight. However, there are several other functions and constraints to which at least some insect wings have to adhere. Both mating behaviour and thermoregulation have been proposed as original functions of structures that would later become wings, and both functions may still be fulfilled by at least some wings today (Alexander and Brown Jr., 1963; Kingsolver and Koehl, 1985; Breitkreuz *et al.*, 2017). Additionally, wings of many species are used for various modes of communication, for example during courtship behaviours. Wings may be used to produce auditory or visual stimuli (reviewed in Schubnel *et al.*, 2021), or possess pheromone glands (for lepidopterans, see Farine, 1983; Birch *et al.*, 1990). Courtship behaviour may even be the reason why *Drosophila* wing shape is not optimized for manoeuvring flight (Ray *et al.*, 2016). Wings also function as sensory organs (see section 2.3.4) and can be involved in mimicry, crypsis, hiding and protection (reviewed in Wagner and Liebherr, 1992; Breitkreuz *et al.*, 2017). They also accomodate a circulatory system with dedicated circulatory organs and potentially even pulsatory organs in the wing itself. The circulation of haemolymph is important for wing maturation, and keeping the wing and its sensory organs alive and pliant. For recent reviews on the wing circulatory system see Pass *et al.* (2015) and Salcedo and Socha (2020).

2.3 Properties of insect wings

In order to understand how insect wings work, this section takes a closer look at the general properties of these complex structures. Insects typically possess four wings, a pair of forewings (primaries) and a pair of hindwings (secondaries, Capinera, 2008a). While most insects have four strongly veined wings, dipteran forewings have a reduced vein pattern (see Stark *et al.*, 1999), and their hindwings have evolved into halteres (e.g. Yarger and Fox, 2016).

2.3.1 Layers of the wing

In their mature state, wings generally consist of veins with purely membraneous parts (cells) between them. Wings are outgrowths of the body, therefore the wing membrane possesses a dorsal and a ventral membrane layer. Initially, haemolymph and epidermis constitute a further layer between the dorsal and ventral part, but during maturation of the wing most of the epidermis performs apoptosis. The mature membrane itself has a complex layer structure with two or three main layers, which themselves are layered (Song *et al.*, 2007; Ma *et al.*, 2017). It is few micrometres thick (Rees, 1975b). The wing can only be used for flight after it has matured.

2.3.2 Gross anatomy of the wing

While it is generally accepted that insect wings only evolved once (Crampton, 1916; Kukalova-Peck, 1978; Engel, 2015, but see Matsuda, 1981), vein fusion and loss (e.g. Dimitrowa, 1927) have led to a variety of vein patterns. Insect wings also come in a number of different shapes. Front and hindwings may have similar or dissimilar shapes. Wings can be long and narrow, fan-shaped or anything in between. They may be branched, fringed, modified into elytra or halteres, or reduced completely (see Capinera, 2008b). Consequently, among winged insects, there are morphologically four-winged and two-winged species. In morphologically four-winged insects, the wings may function as a single unit during flight, resulting in functional two-wingedness (see Grodnitsky, 1995, for more detailed classification). Due to this large variability, species-specific data are necessary for investigations on specific details of insect flight.

Figure 2.2 depicts the wing of a calliphorid fly. Wing veins and cells are named according to the version of the Comstock-Needham system used in McAlpine (1981) and Rognes (1991). In flies, the posterior wing pair has been reduced to halteres, sensory organs for flight control (see e.g. Schneider, 1953; Bartussek and Lehmann, 2016; Yarger and Fox, 2016). The wing base, where the wing attaches to the thorax, is a complex structure of many interlocking parts forming a joint. Three short plates (tegula, basicosta and costagium) form the proximal part of the costal margin of the wing. The next section is the costa, which – depending on species – frames the entire wing or parts of it. Compared to other lineages, flies have a reduced venation pattern. Most of the wing mass is located near the wing base and the anterior margin (leading

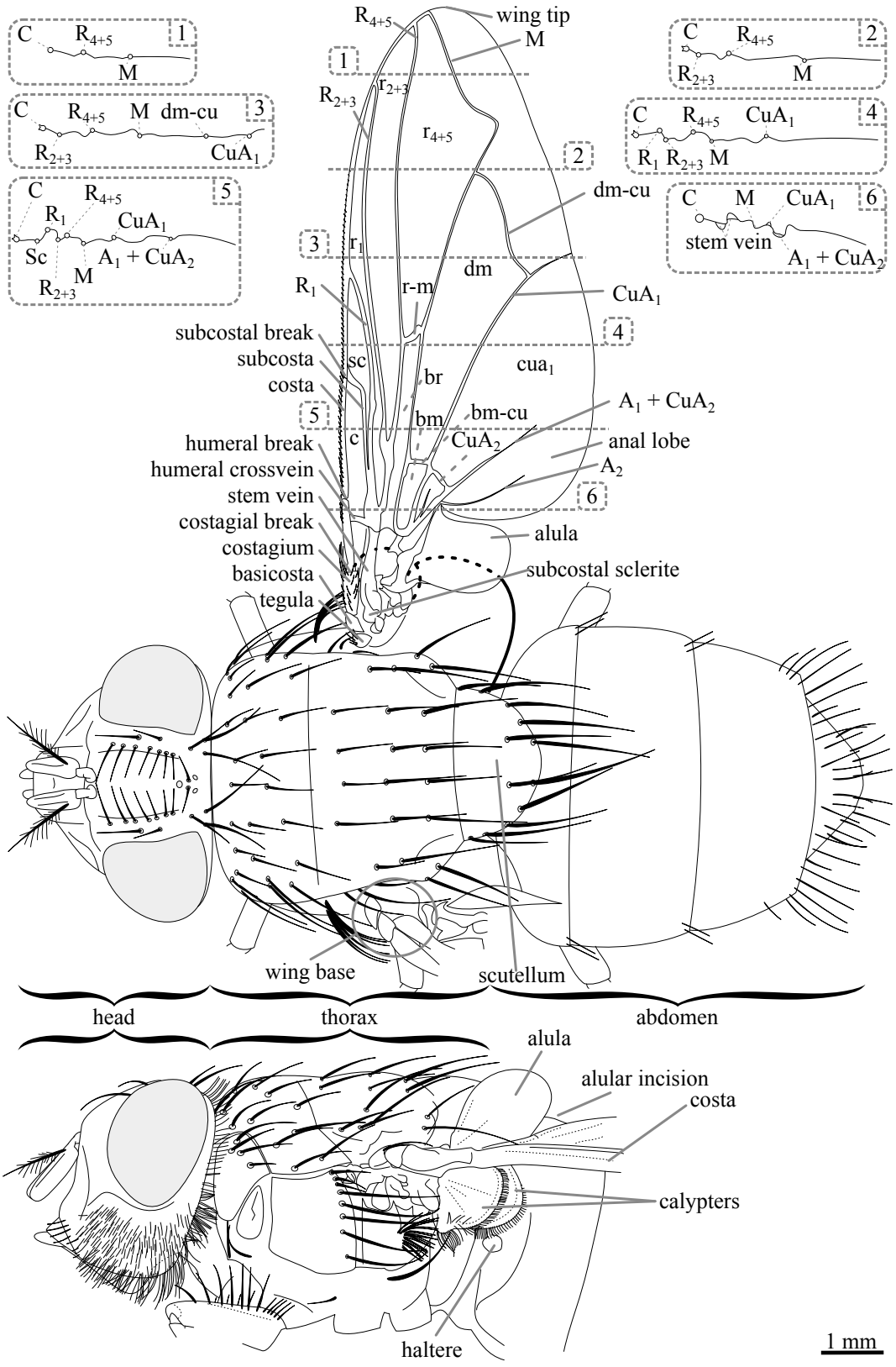

Figure 2.2: Anatomy of a calliphorid fly. Upper: Dorsal view, legs and left wing excluded. Lower: Lateral view, legs, parts of wing, abdomen excluded. C: costa, Sc: subcosta, R: radius, M: media, Cu: cubitus, A: anal vein; cells named like veins, only lowercase (b: basal, d: distal). Insets: Simplified cross-sections of wing at dashed horizontal lines. Body, wing, wing cross-sections: same scale. Membrane thickness not to scale. Cross-sections based on micro-computed tomography scans and profilometer scans of fixed wings, orientation may therefore not be typical.

edge), the location of thick veins (shown for *Calliphora* sp. in Ganguli *et al.*, 2010). Wings are typically rather lightweight, which reduces the inertial power needed during wing flapping (see section 2.2.1): overall, total wing mass amounts to only $\sim 1\,\%$ body mass in flies[2].

Size and shape of wings are important for their aerodynamic properties. Wing length R is the distance between root and tip, along the wing axis (Sane, 2003; Ellington, 1984b). Wing span describes the maximum distance between the wing tips but is often approximated as $2R$ (Sane, 2003; Ellington, 1984b, see also fig. 2.4A). The wing chord c is the distance between anterior and posterior edge of the wing orthogonal to the wing axis (Sane, 2003; Ellington, 1984b). In non-rectangular wings it varies along the wing. The ratio between wing length and average wing chord is the aspect ratio AR of a single flapping wing (Chin and Lentink, 2016). An alternative definition of the aspect ratio is the wing span divided by the mean chord. This definition is meant for fixed-wing fliers according to Chin and Lentink (2016) but is also used for flapping fliers (e.g. Ellington, 1984b). More complex geometrical features of the wing planform are the k-th moments of wing area used by Ellington (1984b). He defines them as $S_k = 2\int_0^R cr^k \mathrm{d}r$. For example, S_0 is total wing area, and S_2 is proportional to lift force, according to Weis-Fogh (1973) as cited in Ellington (1984b, for the definition of lift, see section 2.4.1). Although the aforementioned definition is for two symmetric wings, it is possible to use a modified version to describe single wings, which is useful when dealing with asymmetric wing pairs (Wehmann *et al.*, 2022).

2.3.3 Wing veins

Wing veins are cuticular tubes with different diameters and cross-sectional shapes (summarized in Wootton, 1992). They are important for the mechanical integrity of wings because they stop cracks in the wing from propagating (Dirks and Taylor, 2012). They shape the three-dimensional structure of wings, which influences wing stiffness (e.g. Rees, 1975b). Wing corrugation can easily be seen in wing cross-sections cut perpendicular to the wing axis (see fig. 2.2), but wings can also be cambered (Wootton, 1999; Ning *et al.*, 2017). If a wing is cambered, the central line of its profile deviates from a straight connection between leading and trailing edge (see Böswirth and Bschorer, 2012, p. 247). Camber is usually considered to correspond to larger scales of deviation from a flat plate than corrugation, which is more finely structured (see e.g. Engels *et al.*, 2020).

Joints of different types exist between veins (see Rajabi *et al.*, 2015, for details on different joint types in Odonata). In calliphorid and muscid flies, a prominent joint is located at the humeral break (see fig. 2.2). Wing veins and their joints are important for the mechanical

[2] A single wing of a female *C. vomitoria* weighs less than half a milligramme ($0.36\,\mathrm{mg} \pm 0.10\,\mathrm{mg}$, mean $\pm$ standard deviation, $n = 7$), compared to nearly 60 mg for the entire animal ($59.3\,\mathrm{mg} \pm 6.4\,\mathrm{mg}$, $n = 9$). Similarly, a single wing of a female *M. domestica* weighs only around 60 µg ($0.06\,\mathrm{mg} \pm 0.01\,\mathrm{mg}$, $n = 4$), compared to more than 15 mg for the entire animal ($16.4\,\mathrm{mg} \pm 2.9\,\mathrm{mg}$, $n = 4$). Own data. A previously published study measured 0.24 mg per wing and 59 mg per fly for *Calliphora* sp. (Ganguli *et al.*, 2010).

properties of the wing and its passive deformation during flight (see review by Wootton, 1999). It has actually been shown that different dragonfly wing "modules" do not evolve independently, allowing the wing to keep its functionality for flight (Blanke, 2018). Similarly, Meresman *et al.* (2020) suggest changes in vein patterns may be adaptations to different flight behaviours in beetles.

Apart from mechanical stability, wing veins fulfil further purposes: they carry nerves connected to mechanosensory sensilla (Bomphrey and Godoy-Diana, 2018) and tracheae (Thomsen, 1938). They are also part of the wing circulatory system, which carries haemolymph and keeps the wing alive (see review by Pass, 2018). This circulatory system is also important for wing expansion and maturation and prevents desiccation of the wing and its constituents such as resilin (see Salcedo and Socha, 2020, for another recent review on the wing circulatory system).

2.3.4 Wings and halteres as sensory organs

Insect wings may carry setae or scales (Comstock, 1918, p. 64), some of which are sensory organs (see Pass, 2018). Several different types are located on the wings (Miller, 1970; Dickinson *et al.*, 1997; Yanagawa *et al.*, 2014; Raad *et al.*, 2016; Pass, 2018; Yang *et al.*, 2020). Most of the wing sensory organs, however, are mechanosensors, either for proprioception or for perceiving tactile stimuli, sound or air currents. Hair sensilla on the wing margins are used to elicit scratching or defensive behaviours when touched or to sense vibrations in the air, which is likely important for creating a stable wing beat frequency (Pass, 2018). Campaniform sensilla work as strain gauges and therefore encode movements and deformations of wing joint and wing, making these sensilla important for control of flight and body posture (see reviews by Wootton, 1992; Bomphrey and Godoy-Diana, 2018; Pass, 2018).

In flies, the halteres also possess mechanosensors. Halteres do not have an aerodynamic function and instead function as inertial sensors or gyroscopes. They may share this function with fully developed wings of other species (discussed in Bomphrey and Godoy-Diana, 2018). Haltere sensory inputs and visual stimuli are integrated by the nervous system and used for flight control (Fox *et al.*, 2010; Bartussek and Lehmann, 2016 and reviews by Yarger and Fox, 2016; Dickerson, 2020). The general shape of a haltere is that of a rod or scape with knobs at the base and the tip (Pringle, 1948). In some species, like *M. domestica* or *C. vomitoria*, the halteres are covered by membraneous lobes called calypters. Other species, like *D. melanogaster*, do not have pronounced calypters. Calypters may or may not have an aerodynamic function (Miyan and Ewing, 1985; Dettner and Peters, 2010, p. 875) or simply protect the halteres from air currents (Pringle, 1948).

The halteres beat regularly during flight (Pringle, 1948). They may also beat during walking and other behaviours (see review by Yarger and Fox, 2016). During flight, the halteres appear

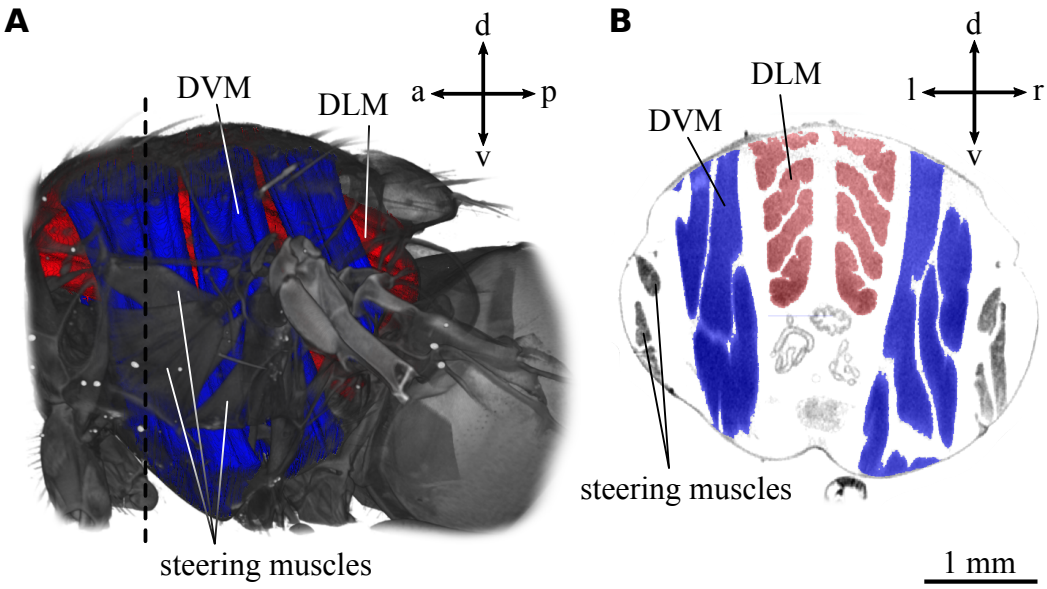

Figure 2.3: Thorax of a female *C. vomitoria* reconstructed from a µCT scan. A: Lateral view. B: Cross section at the position indicated by the dashed line in A. Power muscles marked in red (dorsal longitudinal muscles, DLM) and blue (dorso-ventral muscles, DVM). Some steering muscles are also shown. Compass cards indicate animal orientation; anterior (a) and posterior (p), dorsal (d) and ventral (v), left (l) and right (r).

to beat due to the indirect power muscles, just like the forewings (Schneider, 1953, see also section 2.3.5). Wings and halteres beat at the same frequency and are phase-shifted by a fixed species-specific amount (see review by Yarger and Fox, 2016). Wings, thorax and halteres form a single oscillating system (Schneider, 1953; Deora *et al.*, 2015). Its mechanical setup alone affords a degree of stabilisation against rotations of the fly (Schneider, 1953). When the wing beat frequency is manipulated by clipping the wing, the haltere frequency follows when the changes are small, but larger changes result in the breakdown of the relationship between wing and haltere frequencies (Schneider, 1953; Deora *et al.*, 2021). This may impair flight control in animals with damaged wings.

2.3.5 Flight muscles

For active flight, insects need to beat their wings. As wings do not contain muscles (Wootton, 1999), active wing movement is exclusively achieved by muscles inside the thorax. These flight muscles can be direct (i.e. attached directly to the proximal end of the wing) or indirect (i.e. attached to parts of the thorax). Indirect flight muscles are responsible for deforming the thorax and thereby indirectly exerting forces on the wings (Wendler, 2010, ch. 9.4). In dipterans, indirect dorso-ventral muscles (DVM) and dorsal longitudinal muscles (DLM) are power muscles for wing movement. A number of steering muscles fine-tune the wing motion in flies (Wisser and Nachtigall, 1984; Dickinson and Tu, 1997). See figure 2.3 for a view of the power muscles of *C. vomitoria*.

2.4 Aerodynamics of insect flight

As explained in section 2.2.1, one major challenge for flight is that body weight has to be supported in air, a low-density medium, necessitating high metabolic rates (Schmidt-Nielsen, 1972; Roff, 1990; Dudley, 2000). This invites questions about how insect flight works. Indeed, the widely known bumblebee paradox[3] famously states that according to the laws of conventional fixed-wing aerodynamics bumblebees should be incapable of flight. Bumblebees obviously do fly, and they do this without violating the laws of physics through sheer ignorance. This highlights the importance of using adequate models suitable for the situation under consideration. In the following, a number of terms and basic concepts also used in fixed-wing aerodynamics will be introduced. Then, the principles of flapping insect flight will be discussed.

2.4.1 Basic aerodynamics

Any movement of an object within a fluid, i.e. a gas or a liquid, leads to forces between the object and the surrounding fluid. The resultant forces depend on the relative velocity between object and fluid, the size, shape and orientation of the object relative to the flow, and physical properties of the fluid. These fluid dynamic forces are important in swimming and flying. In order to swim or fly, the weight of the swimmer/flier needs to be supported within a liquid (water) or gas (air), respectively, and directed movement needs to be achieved. In swimming animals, buoyancy often is sufficient for a large part of the weight support, and they need fluid dynamic forces mainly for forwards movement. Fliers, however, usually need to rely on fluid dynamic forces for both weight support and forwards movement (see Schmidt-Nielsen, 1972).

The fluid dynamic force vector acting on an object can be split into two components, lift and drag (e.g. Demtröder, 2021, ch. 8). Both forces depend on the fluid density ρ and a characteristic area A_{char}. The lift force $F_{\text{L}} = \frac{\rho U_\infty^2 A_{\text{char}} c_{\text{L}}}{2}$ (with lift coefficient c_{L}) is oriented perpendicular to the free-stream movement of fluid relative to the object, $\vec{U}_\infty$. For horizontal $\vec{U}_\infty$, the lift force points up and can be used to overcome gravity. The second force, perpendicular to the first, is called drag F_{D}. It is the force "opposing" the relative movement of the object within the fluid and can be calculated as $F_{\text{D}} = \frac{\rho U_\infty^2 A_{\text{char}} c_{\text{D}}}{2}$ (with drag coefficient c_{D}). Drag forces have to be compensated in order to keep the object moving relative to the fluid. In a steady state, the drag force is offset by an equal and opposite force (either via a connection to the ground or via thrust).

Geometry (including texture) and orientation of the object within the flow influence the lift and drag coefficients. These coefficients also depend on Reynolds number (see below). The characteristic area A_{char} is usually the cross-section perpendicular to $\vec{U}_\infty$ for blunt bodies and the wing area or planform area for wings. For details, please refer to textbooks such as Böswirth and Bschorer (2012, ch. 9, 10). Lift and drag both increase with the speed of the object. One

[3] The history of the bumblebee paradox can be found in Sane (2017).

way of describing the efficiency of wings is the lift-to-drag ratio (e.g. Nguyen *et al.*, 2016; Fu *et al.*, 2018). This is due to the fact that for fixed-wing flight in horizontal flows lift forces are desired and drag forces are detrimental.

As explained above, lift and drag are defined by the relative velocity between object and fluid. Depending on context, a different decomposition of the total force may be chosen. It is for example possible to decompose the force acting on a flying animal into a vertical force supporting an animal's weight, a force oriented perpendicular to the vertical force parallel to the animal's median sagittal plane, and a sideways force.

Lift and drag of a simplified fixed wing

The most basic wing or aerofoil is a thin flat plate of infinite length moving through a fluid at a constant velocity. The forces produced by the aerofoil then depend on the angle between $\vec{U}_\infty$ and the chord of the aerofoil. This angle is the (geometric) angle of attack α (Demtröder, 2021, p. 251). For horizontal $\vec{U}_\infty$ and wing, the resultant lift points up. Wings influence the flow around them and the wing may therefore be oriented relative to the local flow at an angle different from the geometric angle of attack. This angle between the chord of the wing and the locally deflected flow is the effective angle of attack α_{eff} (Sane, 2003; Lehmann, 2004b).

In order to calculate lift and drag, the aerodynamic coefficients need to be determined. For complex objects, this often needs to be done experimentally (Böswirth and Bschorer, 2012, p. 216). For thin flat plates or thin symmetric aerofoils, the coefficients can be estimated easily for the two extreme positions. For small angles of attack ($\alpha < 5°$), the lift and drag coefficients can be approximated as $c_{\text{D}} \approx 0$ and $c_{\text{L}} \approx 2\pi \sin(\alpha) \approx 2\pi\alpha$ (see Wang, 2005; Böswirth and Bschorer, 2012, p. 249). For the other extreme position, i.e. for flat plates oriented nearly perpendicular to $\vec{U}_\infty$, there are discontinuities in the fluid velocity at the edges and an area of unmoving fluid directly behind the plate. Drag and lift coefficients can in this case be estimated as $c_{\text{D}} \approx \frac{\pi \sin^2(\alpha)}{4+\pi \sin(\alpha)}$ and $c_{\text{L}} \approx \frac{\pi \sin(2\alpha)}{4+\pi \sin(\alpha)}$. This approach underestimates drag because the pressure is assumed to be the same close to and far away from the plate, which is not true (Wang, 2005).

This way of calculating lift and drag includes several assumptions that may not be met by real objects like insect wings. If true, this simple model may be ill-suited for describing the flight of insects. The flow is assumed to be incompressible (Böswirth and Bschorer, 2012, p. 266). Another assumption is that the fluid is inviscid. Additionally, the wing is considered to be thin, (infinitely) long and moving at a constant velocity and orientation. Insect wings are undoubtedly thin and despite their three-dimensional structure comparatively flat, but the validity of the other assumptions needs to be evaluated.

Can the flow during insect flight be assumed to be incompressible? Often, gases and liquids are distinguished by gases being easily compressed and fluids being incompressible, but de-

pending on the pressure no real fluid is truly incompressible. The question then becomes for which flows incompressibility is a reasonable approximation. The answer is that it is possible to assume incompressible flows in gases up to Mach number $M = 0.3$ for blunt bodies and up to $M = 0.7$ for more narrow ones (Böswirth and Bschorer, 2012, p. 266). The Mach number M is the ratio of a speed, for example of an object moving within a fluid, relative to the respective speed of sound (Spektrum, 1998b). The speed of sound in air at 20 °C and 101.3 kPa is approximately $344\,\mathrm{m\,s^{-1}}$ (Becker *et al.*, 2005, p. 76). Insects and birds tend to operate at $M \approx 0.003$ (Wang, 2005). One of the *M. domestica* individuals from Nasir (2017) had a wing beat frequency $f = 164\,\mathrm{Hz}$, a wing length $R = 6.12\,\mathrm{mm}$, and a maximum and mean wing tip velocity of $8\,\mathrm{m\,s^{-1}}$ and $4.7\,\mathrm{m\,s^{-1}}$, respectively (Thomas Engels, pers. comm.), resulting in Mach numbers of 0.02 and 0.01. It is therefore reasonable to assume incompressible flows when dealing with insect flight.

Can the flow during insect flight be assumed to be inviscid? All real fluids possess some degree of inner friction called the dynamic viscosity μ. It increases with rising temperature for gases while it decreases with rising temperature for liquids (Kuchling, 2004, pp 168f.). Air typically has a dynamic viscosity of $\mu \approx 18\,\mu\mathrm{Pa\,s}$ (Kuchling, 2004, p. 622, Ostadfar, 2016, p. 16, temperatures 0 °C to 20 °C, pressure 101.3 kPa). Another way to describe the viscosity is the kinematic viscosity ν, which is the dynamic viscosity divided by the density of the fluid ($\nu = \frac{\mu}{\rho}$, Kuchling, 2004, p. 169). For air, ν is around $13.3\,\mathrm{mm^2\,s^{-1}}$ at a temperature of 0 °C and a pressure of 101.3 kPa (Kuchling, 2004, p. 622).

The ratio of inertial and viscous forces or viscous dissipation depends not only on the fluid itself but also on the size and speed of the objects interacting with the flow. Flows around objects with similar shape are similar if $\frac{u_{\mathrm{char}} d_{\mathrm{char}}}{\nu} = const.$, where u_{char} is a characteristic speed, usually between object and fluid, d_{char} is a characteristic length, usually the chord of a wing. This ratio is called the Reynolds number *Re* (see reviews by Sane, 2003; Chin and Lentink, 2016, for more in-depth explanations). *Re* can be used to classify the flow regime. For example, increasing the size of an object and using a fluid with matching higher viscosity will result in similar flow patterns, which is useful for enlarged physical models of small objects.

The two extreme flow regimes are laminar and turbulent flow. In laminar flow, the relevant forces are internal friction (viscosity) and pressure gradients. The internal friction forces are large compared to any accelerating forces. One can imagine single layers of fluid sliding across each other without mixing (Spektrum, 1998a, Demtröder, 2021, p. 232, Kuchling, 2004, pp. 167, 169). In turbulent flow, the internal friction forces are small compared to accelerating forces. This results in mixing of the fluid and unsteady flow (see below) even for steady boundary conditions (Spektrum, 1998c, Demtröder, 2021, p. 232, Ostadfar, 2016, p. 19, Chin and Lentink, 2016). All else being equal, higher Reynolds numbers result in more turbulent flow. For insects, usually wing chord and wing tip speed relative to the air are chosen as char-

acteristic length and velocity. Then, typically $10 \leq Re \leq 10000$ holds true, although some very small insects operate at $Re \approx 1$. Diptera usually have Reynolds numbers between 170 and 2000 (Weis-Fogh, 1973). The different orders of magnitude of *Re* for different insects also mean that the same phenomena may work and behave slightly differently in different species (Bomphrey and Godoy-Diana, 2018). For typical insects, both inertial and viscous forces are important (see e.g. reviews by Wang, 2005; Dickinson, 2006; Chin and Lentink, 2016). Consequently, for insect flight, air should not be considered as an inviscid fluid.

The assumption of constant velocity and angle of attack does not hold for flapping insect wings, either. Overall, there are several reasons as to why the above model for thin fixed aerofoils cannot directly be used for describing insect flight. Not only do flying insects – in contrast to fixed-wing aeroplanes – tend to use one and the same structure (their wings) to produce both weight support and thrust (discussed in Bomphrey and Godoy-Diana, 2018), there are ways in which they do not adhere to the assumptions needed by the most basic equations for calculating lift and drag introduced above. For example, insects beat their wings periodically, which means that the wing accelerates and decelerates frequently. Consequently, the assumption of a steady state is not applicable. Additionally, because the wings rotate around the wing joint, there is a velocity gradient along the wing resulting in axial flow. For reviews on the topic of insect flight, see Sane (2003); Wang (2005); Dickinson (2006); Deora *et al.* (2017); Liu *et al.* (2017); Bomphrey and Godoy-Diana (2018).

Descriptions of the flow around wings

If the simple approach above cannot be used to estimate the forces acting on flying insects, more information about the flow around the wings is needed. As discussed in the last chapter, the flow can be assumed to be incompressible, and viscous forces need to be included.

Very generally, flows can be described by characteristic numbers like Mach or Reynolds numbers. Flows can also be classified as steady or unsteady. For steady flows, the velocity field does not change with time, although flow velocities may differ between locations. If the velocity field changes with time, the flow is unsteady. For perpetually accelerating and decelerating flapping wings the flow is inherently unsteady. Some aspects of unsteady flow can be investigated using quasi-steady analysis, which approximates the unsteady conditions as sequential snapshots of steady conditions (Böswirth and Bschorer, 2012, pp 2f.). This approach captures some, though not all, of the factors important for insect flight. For more detailed discussions of the topic, see reviews by Wang (2005); Dickinson (2006). Chin and Lentink (2016) also present several distinct types of quasi-steady approaches.

Mathematically, flows can be described by the changes in velocity of a small volume element of fluid according to time and position. In ideal fluids, the relevant forces are gravity and pressure forces, which are included in the so-called Euler equation. Adding viscous forces

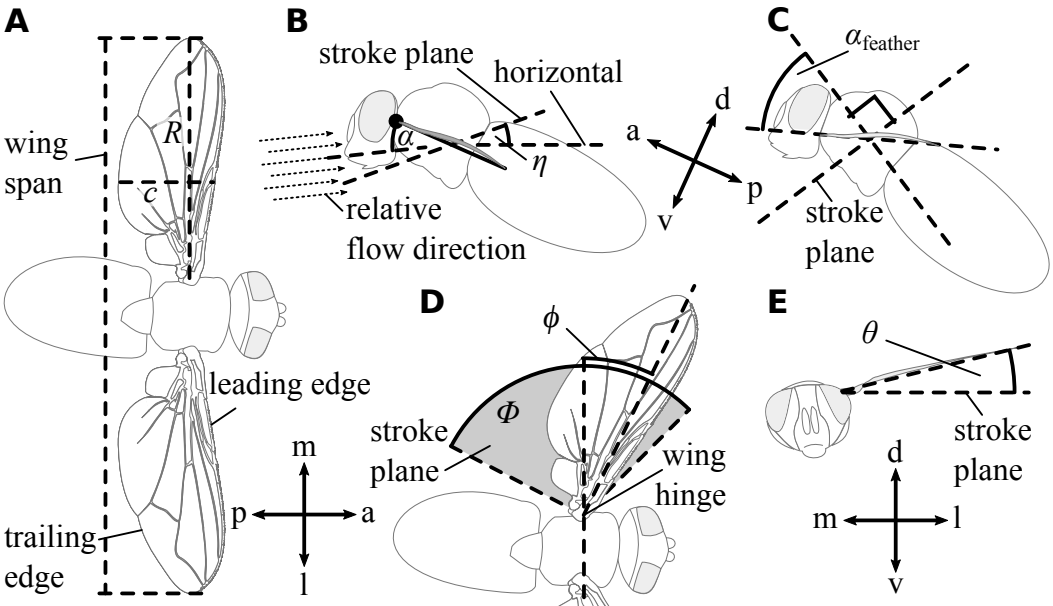

Figure 2.4: Morphological and kinematic terms. Flies are sketched without legs. A: Dorsal view showing wing length *R*, wing span and wing chord *c*. B: Lateral view with stroke plane angle η and angle of attack α. Leading edge and wing chord indicated by "lollipop". The wing is slightly cambered and therefore not completely aligned with the stem of the lollipop. C: Lateral view showing the feathering angle $\alpha_{feather}$. D: Dorsal view showing the positional angle ϕ and the amplitude Φ. E: Frontal view showing the deviation or elevation angle θ. Compass cards give directions relative to animal; anterior (a) and posterior (p), dorsal (d) and ventral (v), medial (m) and lateral (l).

results in the Navier-Stokes equation. This equation and variations of it are widely used to describe and model flows. For further basic information, see Demtröder (2021, ch. 8). Another parameter often used for the description of flows is the vorticity. This is the curl of the flow, and by integrating the vorticity over a wing's surface, a calculation of the aerodynamic forces is possible (Sane, 2003; Lehmann, 2004b; Chin and Lentink, 2016). The Navier-Stokes equation (used for calculating the flow) and vorticity (often used for visualisation and description of the flow) play an important role in computational fluid dynamics (CFD) simulations of insect flight.

2.4.2 Body angles and kinematics

The preceding sections have illustrated that flapping flight, especially at Reynolds numbers typical of insects, cannot be described in a straight-forward manner by the same mathematical models as translating fixed-wing flight of aeroplanes. The motion of a wing during flapping flight constantly changes its orientation as well as the resultant aerodynamic forces. Basic insect wing kinematics will be introduced here, before discussing principles of flapping flight in the next section.

The body of a flying insect can rotate about three axes. Body pitch (angle between anterior-posterior body axis and horizontal), yaw (rotation around vertical axis) and body roll (angle

between left-right body axis and horizontal) can all vary during flight. Body pitch has been found to be 6° to 82° in *C. vomitoria* and 32° to 60° in *D. melanogaster* by Ennos (1989).

When flying, insects rotate their wings around the wing hinge. The stroke plane is an approximation of the plane within which the wing is moved. The angle between the stroke plane and the horizontal is called the stroke plane angle η (see fig. 2.4B). Values for η vary from $-16.5°$ to 47.1° in *C. vomitoria* and from $-10.9°$ to 28.5° in *D. melanogaster* (Ennos, 1989). In the most simple description, the wing performs a downstroke (moving its tip from a dorsal to a ventral position) with the anterior wing edge "leading" the movement. It is then rotated such that the anterior wing edge is still the leading edge during the upstroke (supination), and is rotated again (pronation) at the end of the upstroke (Sane, 2003).

As explained in section 2.4.1, the most important factors for aerodynamic force production are orientation and speed of the wing relative to the flow. The local changes in the flow due to the presence of a wing are often unknown and hard to measure in insects. Therefore, the geometric angle of attack is commonly used (Sane, 2003). In insects, the angle of attack can easily reach maximum values between 25° and 45° (Ellington, 1984c; Sane, 2003; Wang, 2005; Chin and Lentink, 2016). An alternative description of how the wing is oriented within the flow is the feathering angle α_{feather}. It is equivalent to the geometric angle of attack for insects hovering in quiescent air and is defined as the angle between chordwise wing orientation and the perpendicular of the stroke plane (see e.g. Engels *et al.*, 2016b, see also fig. 2.4C). The feathering angle is usually positive during the downstroke and negative during the upstroke, while the angle of attack is defined as positive in both cases.

The average speed of the wing depends on the distance it travels per wing beat and the wing beat frequency f. For fly species investigated for this thesis, the flapping frequency ranges from 120 Hz to 230 Hz (Ennos, 1989). For comparison: the highest reported wing beat frequencies were up to 1 kHz in small dipterans (Sotavalta, 1952b, 1953). Odonata exhibit flapping frequencies of 15 to 40 Hz (Sunada *et al.*, 1998) and flies usually flap between 100 and 250 times per second (Nachtigall, 1966; Ennos, 1989; Lehmann *et al.*, 2011). In cases where the wing stays mostly within the stroke plane, i.e. the deviation angle θ (also called elevation angle; Fry *et al.*, 2005; Liu and Aono, 2009; Engels *et al.*, 2016b, see fig. 2.4E) is small at all times, mean wing tip speed can be approximated as $\bar{u}_{\text{tip}} = 2Rf\Phi$ (fig. 2.4D). Wing beat amplitude Φ is 123° to 150° for *C. vomitoria* and 136° to 162° for *D. melanogaster* (Ennos, 1989).

The instantaneous speed of a flapping wing varies because the wing has to decelerate and accelerate before and after the stroke reversals. The angle of the wing axis within the stroke plane is called the stroke angle with respect to the point of wing pronation (Fry *et al.*, 2005) or positional angle with respect to the left-right body axis (Engels *et al.*, 2016b). It is denoted ϕ (see fig. 2.4D) and has been found to vary in a sinusoidal fashion over the course of a wing beat for *D. melanogaster* (Fry *et al.*, 2005; Dickson *et al.*, 2008).

Specific kinematic measurements have been published for a variety of species. Among them are *Drosophila* sp. (Ennos, 1989; Lehmann and Dickinson, 1998; Bergou *et al.*, 2010; Muijres *et al.*, 2017), *M. domestica* (Nasir, 2017), or *Calliphora vicina* (Nachtigall, 1966; Ennos, 1989; Balint and Dickinson, 2001). Insects can vary their wing kinematics in order to manoeuvre, potentially with every single wing beat and at least in certain aspects for left and right wings independently (Sane, 2003). The larger the forces they produce, the less variable the wing beat kinematics become, as has been shown for *Drosophila* (Lehmann, 2004a).

There are also some more general descriptors of flapping flight, for example the advance ratio J. It is defined as the ratio $\frac{U_\infty}{u_{\text{wing}}}$ between the overall velocity of the flier relative to the air and u_{wing}. The latter is the speed of the wing relative to the body (Wang, 2005), usually $\bar{u}_{\text{tip}}$ (Chin and Lentink, 2016). According to Chin and Lentink (2016), the best propulsive efficiency can be achieved for $1.25 \leq J \leq 2.5$. Birds and insects tend to use advance ratios of $0 \leq J \leq 5$ (Chin and Lentink, 2016; Lehmann, 2004b). For *C. vomitoria*, Ennos (1989) found a body pitch of 6° at an advance ratio of $J = 0.32$ and a body pitch of 82° during hovering.

2.4.3 Principles of flapping flight

Flapping fliers make use of different aerodynamic principles peculiar to the unsteady nature of flapping flight, which together provide a solution for the bumblebee paradox. One of the most famous works on the analysis of insect flight is an article series by Ellington. He developed a way to calculate the average values of several key parameters such as lift for hovering insects but cautioned that he did not expect the quasi-steady approach to be completely valid (Ellington, 1984a). The computational power available today and advances in flow visualisation techniques have enabled researchers to use ever more detailed models for the study of insect flight. These may, for example, include detailed unsteady and/or turbulent flows, complex geometries and fluid-structure interactions. Models can either be physical (e.g. Kesel, 2000; Tanaka *et al.*, 2011; New *et al.*, 2014; Moses *et al.*, 2017; Fu *et al.*, 2018) or based on computational fluid dynamics (e.g. Luo and Sun, 2005; Meng and Sun, 2011; Chen and Skote, 2016). Despite having grown ever more realistic, they are still simplified, partly by necessity and partly in order to ask specific questions. For example, wing models are often stiff instead of flexible (e.g. Luo and Sun, 2005; Meng and Sun, 2011; Barnes and Visbal, 2013), flat instead of corrugated (Zheng *et al.*, 2013; Engels *et al.*, 2016a; Ravi *et al.*, 2016), or use simplified wing kinematics (e.g. Sane, 2003; Bhat *et al.*, 2019). Sometimes models are (functionally) two-dimensional (e.g. Vargas *et al.*, 2008; Jain *et al.*, 2015; Flint *et al.*, 2017; Ansari and Anwer, 2018), and many studies have been done on gliding instead of flapping flight (e.g. Kesel, 2000; Vargas *et al.*, 2008; New *et al.*, 2014; Ansari and Anwer, 2018). Despite the complexity of the problem, several of the unsteady principles underlying insect flight aerodynamics are understood quite well today. Some prominent ones will be introduced here.

A strategy enhancing lift employed by some insects is the clap-and-fling or clap-fling-flip mechanism. It was first described by Weis-Fogh (1973), and has been reviewed in detail (e.g. Sane, 2003; Lehmann, 2004b). The "clap" refers to the wings (nearly) coming into physical contact, leading edge first, at the dorsal stroke reversal. This might reduce the amount of vorticity shed by the wings. The clap also pushes air out between the wings, which propels the animal forward. The propulsive efficiency can be improved through wing flexibility as has been shown for butterflies, where the "clapping" produces thrust and the downstroke produces lift (Johansson and Henningsson, 2021). The "flinging" apart of the wings is thought to facilitate the creation of new vorticity attached to the wings (Weis-Fogh, 1973; Sane, 2003; Lehmann, 2004b). The wings also "flip" at the stroke reversals, inspiring the name clap-fling-flip used by Weis-Fogh (1973). Overall, the clap-and-fling is thought to slightly improve lift (around 25 % muscle mass-specific lift, Marden, 1987) and provide some forward propulsion. It is not used by all insect species or all the time and appears to be mainly restricted to scenarios like carrying heavy loads or flying tight curves, where it may be employed to maximize the wing beat amplitude rather than the specific benefits of the clap-and-fling itself (Sane, 2003; Lehmann, 2004b; Chin and Lentink, 2016).

Another unsteady effect is the Kramer effect. When the wings pronate or supinate, a sharp velocity gradient is created at the trailing edge, resulting in shear forces and additional rotational circulation around the wing (reviewed in Sane, 2003). This creates additional forces, the magnitude and direction of which depend on the exact timing and duration of the wing rotation as well as on the location of the rotation axis (discussed in detail in Chin and Lentink, 2016).

Of great importance for lift production in insects is a stable leading edge vortex (Ellington *et al.*, 1996). At the sharp leading edge of insect wings operating at high angles of attack, flow separation occurs and a vortex attached to the wing is created, which is called the leading edge vortex (LEV). It creates a suction force that acts in a direction vertical to the wing surface, creating both lift and drag (see e.g. Sane, 2003; Lehmann, 2004b). This may be theoretically less efficient than creating lift at lower angles of attack without using a stable LEV, but the latter strategy would necessitate physiologically impossible flapping frequencies (Dickinson, 2006). While the exact structure of the LEV is dependent on Reynolds number and its stability may be influenced by the advance ratio (Liu *et al.*, 2017), overall it is an effective mechanism. Especially in flapping wings, spanwise flow stabilises the LEV by limiting its growth (Wang, 2005; Eldredge and Jones, 2019). The stable LEV appears to be quite robust against turbulence (Bomphrey and Godoy-Diana, 2018; Matthews and Sponberg, 2018) and has been found in invertebrates, vertebrates and even plants, indicating that it is a convergent solution to the task of lift production (see reviews by Chin and Lentink, 2016; Liu *et al.*, 2017).

At the stroke reversals, the LEV and its counterpart, the trailing edge vortex, are shed from the wing into the wake. A wing moving back and forth may interact with its own wake, which

changes the aerodynamic forces on the wing in a complex way. The exact effect of wing-wake interaction on the forces acting on the wing depends for example on wing kinematics (for details, see reviews by Sane, 2003; Lehmann, 2004b; Dickinson, 2006; Lehmann, 2008)

Insects vary their wing beat kinematics (and body posture) in order to manoeuvre and steer, or for stabilisation (reviews: Combes and Dudley, 2009; Beatus *et al.*, 2015; Berthé and Lehmann, 2015; Crall *et al.*, 2017; Liu *et al.*, 2017; Bomphrey and Godoy-Diana, 2018; Hamid, 2022). This is an important ability because natural flows tend to be non-uniform. Despite the fact that often the length scale of turbulence is considerably larger than the insect and flapping flight is rather robust against disturbances, insects do react actively to turbulence and wind, which is metabolically costly (reviewed in Liu *et al.*, 2017). This is possible due to sophisticated flight control mechanisms. For reviews on this topic, see Taylor (2001); Dudley (2002); Sun (2014); Dickinson and Muijres (2016). For successful active flight control, sensory input and motor output are needed. The main sensory organs playing a role in flight control in flies seem to be the eyes, antennae and halteres (Dickinson and Muijres, 2016).

While the kinematics used by individual animals can be measured comparatively easily using high-speed cameras, the various vortex-based and unsteady aerodynamic phenomena are hard to measure in actual insects and hard or impossible to calculate analytically. Models, especially CFD models, can help to determine the forces and flows relevant for insect flight, despite some inevitable simplifications and uncertainties. Models also have the advantage that researchers can control and modify them more easily than real animals.

2.5 Aim of the thesis

While both the origin of insect wings (see section 2.2) and the aerodynamic principles of insect flight (see section 2.4) are broadly known, the different constraints and evolutionary pressures acting on complex insect wings (see section 2.3) mean that detailed investigations into how wings function in flight (see section 2.4) need species-specific data and models. The aim of this thesis was to procure such data. In order to investigate the influence of corrugation on aerodynamics, stiff wing models based on micro-computed tomography were created and used in CFD simulations (Engels *et al.*, 2020). Another part of the project focused on measuring the stiffness of real fly wings in order to add to the existing knowledge on insect wing flexibility and to allow fluid-structure interaction in future wing models (Wehmann *et al.*, 2019). The last part of the project was on wing damage, which accumulates in insects and may affect their flight performance. Data on age, activity, wing damage, and flight ability was collected with the aim of evaluating the influence of age and activity on wing damage accumulation, and the influence of age and wing damage on flight ability (Wehmann *et al.*, 2022). Another goal was to provide information for use in future experiments on the influence of wing damage on insect flight aerodynamics.

Chapter 3

Summary and discussion of the studies

The three studies included in this thesis focus on different properties of insect wings relevant for flight in three different species of fly. The work presented here may also be used as a basis for detailed species-specific wing models in future research on insect aerodynamics. After a brief summary of all studies, each will be discussed in-depth.

3.1 Summary of the studies

3.1.1 Summary of study 1 on insect wing corrugation and aerodynamics

There has been an ongoing debate about whether or not corrugation directly influences the aerodynamic properties of insect wings. The aim of the study "Three-dimensional wing structure attenuates aerodynamic efficiency in flapping fly wings" (Engels *et al.*, 2020, included in this thesis, see p. I) was to investigate the direct influence of three-dimensional structures of different size scales on the aerodynamic properties of insect wings. The chosen approach was to create three-dimensional wing models based on real fly wings, modify them to include or exclude small-scale (corrugation) or large-scale (camber) structures and to perform CFD simulations. The data on which the models were based was collected as part of the work for this thesis. Micro-computed tomography scans were performed on the wings of three species (*C. vomitoria*, *M. domestica*, *D. melanogaster*). The reconstructed scans were manually segmented (using the program Icy, de Chaumont *et al.*, 2012) and exported as three-dimensional binary image stacks. Due to issues with overlapping wing membranes, the models were supplemented with profile data from Wehmann *et al.* (2019) where necessary. Final creation of the models was not part of this thesis. In brief, each wing model consisted of a central profile with thickness information at each point. Fourier analysis was used to exclude small-scale and/or large-scale spatial frequencies to create a set of four models (natural, camber-only, corrugation-only, flat). Generalized fly wing kinematics (following Lehmann *et al.*, 2011) with different wing beat fre-

quencies for each species and an advance ratio of $J = 5$ were used for the simulation of fluid dynamics around the wing with FluSi[1], which has been specifically developed for simulations of flapping insect flight (Engels *et al.*, 2016b). For the range tested (*Re* on the order of 10^2 to 10^3), no consistent dependency on Reynolds number was found for the impact of corrugation and camber. Camber acted like a change in geometric angle of attack, and it appeared to reduce flight efficiency. Corrugation did not change the aerodynamics much and no trapped vortices were observed in the corrugation valleys. For the bigger fly species, regions of high pressure differences between ventral and dorsal wing side appeared to be aligned with wing veins. Overall, the investigation of the direct influence of three-dimensional structures of insect wings on their aerodynamics was performed successfully.

3.1.2 Summary of study 2 on wing flexibility

Insect wings are mechanically very complex structures and species-specific data is important for detailed investigations of insect wing aerodynamics. The aim of the study "Local deformation and stiffness distribution in fly wings" (Wehmann *et al.*, 2019, included in this thesis, see p. II) was to determine wing flexibility for three fly species in order to add to the existing data on wing flexibility and as a basis for flexible wing models for further aerodynamic research. The chosen approach was to measure deformation and wing stiffness distribution in living flies. Wings of living flies were extended laterally and immobilized. Point forces of the order of up to one mN for *C. vomitoria* and few µN for *D. melanogaster* were applied to different positions on the wings and deformation was measured statically using an optical profilometer. Wing stiffness increased with increasing animal size. Median spring stiffness values across all measurements was $0.04\,\mathrm{N\,m^{-1}}$ for *D. melanogaster*, $0.14\,\mathrm{N\,m^{-1}}$ for *M. domestica* and $0.36\,\mathrm{N\,m^{-1}}$ for *C. vomitoria*. Wing tip and trailing edge were more flexible than areas with strong venation. For two species (*D. melanogaster* and *C. vomitoria*) no dorso-ventral anisotropy in stiffness was found, while *M. domestica* wings seemed to be more flexible when forces were applied on the dorsal wing side. Veins appeared to structure the wings' deformation pattern. Overall, wing stiffness was successfully measured in all three species, but one limitation for use in artificial wing models is that static measurements were used.

3.1.3 Summary of study 3 on wing damage progression

Over time, insect wings accumulate damage, which may influence flight performance. The exact relationship between age, flight activity and damage progression in single individuals is not yet completely understood. The aim of the study "Flight activity and age cause wing damage in house flies" (Wehmann *et al.*, 2022, included in this thesis, see p. III) was to investigate whether age or activity is responsible for wing damage and how age and wing damage influence flight

[1] https://github.com/pseudospectators/FLUSI

ability. The chosen approach was to collect data on age, wing damage, and activity or flight ability throughout the lives of individual flies. Wing state was recorded using photos. For some animals, kept in small containers in groups of four, sound recordings served to estimate average activity of individuals. Other animals, kept in flight cages, were used to investigate flight ability over the animals' lifetime. Animals in the flight cage lived longer than ones kept in small containers. Females lived longer than males and males were more active than females. Wing damage usually initially appeared at the distal part of the wing. Once damage occurred, it increased exponentially before tapering off, resulting in the progression of wing damage following a logistic curve. Both age and activity were found to predict wing damage, but their relative influence could not be determined and there was strong interindividual variability. The rate at which damage progressed did not appear to depend on the age or activity at which the damage first started. The data also indicate that flight ability decreases with increasing age, wing damage levels, and wing damage asymmetry. Overall, the initial questions could not be answered completely, but the resultant data set on wing damage progression is well-suited to serve as a basis for further experiments.

3.2 Discussion of the studies

3.2.1 Insect wing corrugation and aerodynamics

The study discussed here is "Three-dimensional wing structure attenuates aerodynamic efficiency in flapping fly wings" (Engels *et al.*, 2020, included in this thesis, see p. I).

Insect wings are corrugated (Rees, 1975b), which is important for their mechanical properties (Wootton, 1981, 1993; Kesel, 2000; Lian *et al.*, 2014; Rajabi *et al.*, 2016). Wing flexibility for example allows wing camber to change over the course of a wing stroke (Le *et al.*, 2013), but there has been a long-standing discussion on the topic of whether or not corrugation directly influences the aerodynamic properties of the wing in a meaningful way. The goal of this study was to approach the problem by looking at the wings of specific insect species.

Influence of three-dimensional structure on wing aerodynamics

Significant influence of the three-dimensional structure of wings on their aerodynamics has been found in many studies, but some claim an improvement in performance (Okamoto *et al.*, 1996; Kesel, 2000; Kim *et al.*, 2009; Jain *et al.*, 2015; Chen and Skote, 2016; Feaster *et al.*, 2017; Ansari and Anwer, 2018), while a few find the opposite (Okamoto *et al.*, 1996; Hord and Liang, 2012; Meng and Sun, 2013; Ansari and Anwer, 2018). One particular simplified dragonfly wing profile used in two studies was even found to have better aerodynamic properties in gliding flight than both a flat plate and a more realistic dragonfly profile by New *et al.* (2014) but when oscillating exhibited lower propulsive efficiency than smooth profiles (Flint *et al.*,

2017). Some authors are unconvinced of any meaningful aerodynamic effect (Rees, 1975a; Luo and Sun, 2005; Meng and Sun, 2011; Le *et al.*, 2013; Shahzad *et al.*, 2017), or conclude that effects depend on Reynolds number (Vargas *et al.*, 2008; Murphy and Hu, 2010; Meng and Sun, 2013).

The existence of trapped air within the valleys of wing corrugation has been proposed as one possible explanation for better aerodynamic properties due to wing corrugation, either as stagnant air or in the form of small vortices, making the wing act like one with a smooth profile. The concept was proposed at least as early as Rees (1975a), who suggested that trapped air might allow the wing to be lightweight but still stiff and also function like a smooth aerofoil. Kesel (2000) used physical models based on cross-sections of a dried dragonfly wing at $Re \approx 10000$ and found increased lift compared to a flat plate. She attributed this to vortices in the corrugation valleys. While Vargas *et al.* (2008) questioned the results obtained by Kesel (2000) based on her experimental setup, other studies could indeed show the existence of vortices or "circulation bubbles" in corrugation valleys at high Reynolds numbers using particle imaging velocimetry ($14000 \leq Re \leq 125000$; greater than Re at which insects operate; Murphy and Hu, 2010; New *et al.*, 2014). These went hand in hand with higher lift in the corrugated profile compared to a flat plate and a smooth profile for $Re \leq 100000$ (Murphy and Hu, 2010). For lower Reynolds numbers more typical of insect flight ($150 \leq Re \leq 10000$), a simulation also found such vortices, but with decreasing Reynolds number, the vortex strength decreased (Barnes and Visbal, 2013).

Indeed, it seems likely that the aerodynamic effect of corrugation depends on the Reynolds number. At Reynolds numbers much higher than those of typical insects, Murphy and Hu (2010) compared physical models of gliding wings. At $Re = 58\,000$, the corrugated profile (based on a profile used by Vargas *et al.*, 2008, which in turn is similar to one of the profiles in Kesel, 2000) showed higher lift coefficients than either a flat plate or a profiled wing, while at $Re = 125\,000$ the profiled wing had the highest lift coefficients. At lower *Re*, Meng and Sun (2013) compared five symmetrical profiles with triangular wave corrugation based on Rees (1975b) and one flat wing for $200 \leq Re \leq 2400$. They used CFD simulations of gliding flight and found reduced lift for the corrugated profiles for $15° \leq \alpha \leq 25°$. The effect was less pronounced for small *Re* (between 8 % and 14 % at $\mathrm{Re} = 200$) than for large *Re* (between 13 % and 24 % at $Re = 1000$ and $Re = 2400$). In the same vein, the ratio $c_\mathrm{L}/c_\mathrm{D}$ of three-dimensional gliding dragonfly wing simulations was relatively similar for profiled and corrugated models at $Re = 1400$ (peaking at 3.8 for the profiled wing and 3.6 for the corrugated wing), while the difference was larger for $Re = 10000$ (peaking at 5.9 for the profiled wing and at 4.6 for the corrugated wing, Chen and Skote, 2016). From this, it may be concluded that little influence of corrugation on wing aerodynamics at the low Reynolds numbers of flies can be expected.

Conceivably, small structures like corrugation are less important for the aerodynamic properties of a wing than larger structures like camber. Luo and Sun (2005) argued that corrugation is much smaller than the LEV or region of flow separation, and as such should not be able to influence them greatly. An aerodynamic influence of camber has been observed by Le *et al.* (2013) for a beetle and by Okamoto *et al.* (1996) for a dragonfly. Feaster *et al.* (2017) also found for a bumblebee that larger scale structures are more important than structures on a smaller scale.

The angle of attack as an important factor for lift production is a valid parameter in both gliding and non-gliding flight and also appears to influence how important corrugation is for wing aerodynamics. The three-dimensional gliding dragonfly-based wing simulation by Chen and Skote (2016) found at $\alpha = 5°$ and $Re = 1400$ similar lift and less drag compared to a profiled wing (ca. 6 % higher lift-to-drag ratio), while at $\alpha = 10°$, the profiled wing produced 3 % less drag than the corrugated wing but similar lift, and at $\alpha = 40°$ both wings showed the same performance. Compared with a flat plate, however, a gliding symmetrical triangular wave profile had lower lift coefficients at $Re = 200$ (Meng and Sun, 2013). The difference was small for small angles of attack ($\alpha = 5°$, $c_{\mathrm{L,\,flat}} = 0.27$, $c_{\mathrm{L,\,corrugated}} = 0.24$) and increased for bigger angles of attack ($\alpha = 15°$, $c_{\mathrm{L,\,flat}} = 0.67$, $c_{\mathrm{L,\,corrugated}} = 0.58$), before decreasing again ($\alpha = 25°$, $c_{\mathrm{L,\,flat}} = 0.74$, $c_{\mathrm{L,\,corrugated}} = 0.69$). It also appears that high angles of attack prevent the formation of trapped vortices in the corrugation valleys in beetle (three-dimensional flapping CFD model, Le *et al.*, 2013) and dragonfly wings (three-dimensional gliding CFD model, Barnes and Visbal, 2013). Considering that the angle of attack changes over the course of a wing beat, wing kinematics are likely to influence the magnitude of aerodynamic effects of wing corrugation.

As discussed in section 2.4.3, spanwise flow influences the LEV and thereby lift production. Observations of such flow also exist in studies on the aerodynamic influence of the three-dimensional structure of the wings (Shahzad *et al.*, 2017, same dragonfly model as in Murphy and Hu, 2010). Especially for non-gliding flight, three-dimensional models are usually preferable (see also Le *et al.*, 2013, for comparative two- and three-dimensional CFD models of beetle wings). Notably, effects of corrugation are predominantly seen in studies on gliding rather than non-gliding flight. Many studies using gliding flight find effects of corrugation at least under certain conditions: most of them are studies on models based on dragonfly wings (e.g. Okamoto *et al.*, 1996; Kesel, 2000; Vargas *et al.*, 2008; Murphy and Hu, 2010; Hord and Liang, 2012; Chen and Skote, 2016; Ansari and Anwer, 2018), but Xiang *et al.* (2016) used locust models, and models less strictly based on any particular species were employed by Meng and Sun (2013) and Jain *et al.* (2015). Studies using non-gliding models, however, do not usually report definite effects of corrugation on performance (bumblebee: Meng and Sun, 2011; hoverfly: Du and Sun, 2012; beetle: Le *et al.*, 2013; dragonfly: Shahzad *et al.*, 2017).

Models and hypotheses used in the study

The literature shows that when considering the importance of three-dimensional wing structures for wing aerodynamics many factors play a role. Any effects depend on Reynolds number, size of the structures (corrugation, camber) and kinematics. In order to add to the discussion in a meaningful way, the focus of the study in Engels *et al.* (2020) was to investigate the effect of corrugation and camber in flapping flight of insects from within the same order flying at different Reynolds numbers ($Re = 137$ for *D. melanogaster*, $Re = 892$ for *M. domestica*, $Re = 1623$ for *C. vomitoria*, based on mean wing chord and $\bar{u}_{\text{tip}}$).

A CFD approach was chosen because it allowed total control of the models and advanced calculation of the flow around the wings. Details of corrugation and camber appear to matter (Okamoto *et al.*, 1996), therefore both needed to be as close to the real wing as possible. While effects of aspect ratio and wing planform seem to be small at least in flat plate wings (Luo and Sun, 2005, but see discussion in Krishna *et al.*, 2020), adding a realistic wing planform or contour was no obstacle for a model fulfilling the above criteria. Therefore, this was also included. Using computational models allowed the creation of four different versions of each wing, containing either small-scale corrugation and large-scale camber, only camber, only corrugation, or neither camber nor corrugation.

Wing models were based on data from micro-computed tomography. The reconstructed wing scans were manually segmented to create three-dimensional wing models using software by de Chaumont *et al.* (2012). The thinner parts of the wing were not well-resolved and the posterior margins of the two wings tended to overlap, making it difficult to accurately tell them apart. Where it was necessary, the trailing edge of the models was replaced by membrane of minimum wing thickness according to optical profilometer scans from Wehmann *et al.* (2019). Neither tomography nor profilometer scans were performed on freely behaving animals, wing conformation may therefore not be identical to a normal resting conformation. Due to the fact that conformation is variable during flight, this was deemed acceptable.

While wing shape was chosen to be as realistic as possible, other simplifications were made. Wing flexibility leads to the wing changing conformation over the course of a wing beat (Wootton, 1999). Including conformational changes in the models would have made it hard to tell apart effects of shape and of changes in shape. Therefore, stiff wing models were used. In order to allow easier comparison of the different Reynolds numbers, the same generic fly kinematics were used for all three species. Both of these simplifications allow better comparison of results, but they make the resultant aerodynamic forces less likely to be exactly correct for each species. Due to numerical constraints, minimum wing thickness of the models had to be increased to roughly 28 µm (*D. melanogaster*), 75 µm (*M. domestica*) and 114 µm (*C. vomitoria*). A further simplification was to use a single wing and omit the body of the fly. The focus was on the aerodynamic properties of the wing and not the entire insect, therefore including the body and second wing was not necessary.

The use of non-simplified corrugation, camber and planform in models is still unusual. Studies notable for using rather realistic models include Meng and Sun (2011), who, although removing camber and using triangular waves for the corrugation on rectangular wings, used bumblebee kinematics for their flapping models and set the aspect ratio of the wings to be close to bumblebees as well. Luo and Sun (2005) used accelerating wing rotation, corresponding to the beginning of a half-stroke, with wings of different insect planforms and triangular wave corrugation. Le *et al.* (2013) used wing kinematics, corrugation and camber from a beetle and discussed the change in camber between up- and downstroke. None of these studies found a pronounced influence of corrugation on the aerodynamic properties of the wings, even though Luo and Sun (2005) observed local changes in pressure. Le *et al.* (2013) also included camber and did find a significant influence of this property on aerodynamic performance. The initial hypothesis therefore was that there would be little difference between the overall aerodynamics of fly wing models with and without corrugation. Camber, however, was expected to have a more pronounced influence. A Reynolds number effect was potentially expected.

Contextualization of results

As expected, corrugation appeared to have less of an effect on wing aerodynamics than camber, which acted as a change in angle of attack. The lack of influence of Reynolds number observed in Engels *et al.* (2020) may be due to the relatively small range used compared to other studies (see above) and the fact that there were only three distinct *Re*-values in the study. Another possible explanation is offered by differences in wing shape between species making it more difficult to detect Reynolds number effects, despite the apparently small effects of wing planform (Luo and Sun, 2005). Taking into account that the models were stiff, where real wings are flexible, differences in camber in the models (attributable either to species or to the conformation during the scan of the specific fly wings used as a basis for the models) may also have had an influence, which may overpower any consistent effects of Reynolds number.

Similar to the observation of high pressure differences between ventral and dorsal wing side aligning with veins in Engels *et al.* (2020, even in flat models), the edge of the region of low pressure due to the LEV seems to be aligned with the border between fore- and hindwing in a bumblebee simulation shown in Liu *et al.* (2017) and with wing veins in a moth model pictured in Nakata and Liu (2012). Simulations of locust wings by Young *et al.* (2009) show a less pronounced alignment. For a *D. melanogaster* simulation, no obvious alignment appears (Ramamurti and Sandberg, 2007). This is in line with the least apparent pattern being observed for this species in Engels *et al.* (2020). Considering the fact that veins increase mechanical stability (e.g. Sunada *et al.*, 1998; Rajabi *et al.*, 2016), it seems plausible that areas with the highest pressure difference between ventral and dorsal side would benefit from structural reinforcement provided by veins. Consequently, alignment between ventral/dorsal pressure differences and wing veins would be expected. Rees (1975b) already suggested that corrugation, and the cor-

responding increase in stiffness (see section 3.2.2 below), is strongest in the wing half closest to the leading edge because that is where the strongest aerodynamic forces attack. Future investigations on the alignment between wing veins and regions of strong ventral/dorsal pressure differences during the wing stroke may be worthwile in order to test this hypothesis further and explain why such an alignment is visually apparent in some species but not others.

In sum, the study (Engels *et al.*, 2020) could show that wing corrugation has no significant direct impact on the aerodynamics of fly wings, and is therefore more likely to indirectly impact wing aerodynamics via wing flexibility. Wing camber, however, was found to be important. It is worth noting that camber changes dynamically over the course of a wing beat. Detailed investigations of the aerodynamic significance of wing camber, or whether wing vein patterns have evolved to specifically support regions of the wing with high ventral/dorsal pressure differences should therefore ideally include wing deformation. One way to model conformational changes during flight is to include wing flexibility in the model.

3.2.2 Insect wing flexibility

The study discussed here is "Local deformation and stiffness distribution in fly wings" (Wehmann *et al.*, 2019, included in this thesis, see p. II).

Insects can only actively move their wings using muscles inside the thorax, because the wings do not possess internal muscles (see section 2.3.5). Consequently, most wing deformation during flight is passive, and areas of different stiffness serve to control wing shape in a way that is not deleterious, or even advantageous, to flight (e.g. Wootton, 1999; Rubio *et al.*, 2018). Therefore, wing flexibility is an important aspect of insect aerodynamics. In order to add to the overall data available on wing stiffnes of various species and to be able to expand the models used in Engels *et al.* (2020), the goal of this study was to investigate wing flexibility in the same three species.

Wing flexibility and aerodynamics

Insect wings need to be stiff in order to withstand aerodynamic forces, and corrugation achieves an increase in stiffness without adding much mass to the wing, which would increase the inertial power required (Rees, 1975b). Corrugation creases are typically organized along the wing length, which increases spanwise bending stiffness but easily allows torsion and wing camber (Sunada *et al.*, 1998; Rajabi *et al.*, 2016). The anisotropy between wing length and wing chord allows wing torsion, which typically appears at the stroke reversals (Ning *et al.*, 2017). The corrugation at the leading edge of the wing can also result in an asymmetry between up- and downstroke, as shown for butterflies by Wootton (1993). According to Wootton (1981), stiff wing areas are characterized by strong, sometimes fused, veins, pronounced three-dimensionality and

thick membrane. In contrast, more flexible areas tend to possess less or no veins, little corrugation, no or weak cross-veins, and flat membraneous parts with flexion lines. The membrane between veins can itself increase stiffness and integrity of the wing (Newman and Wootton, 1986; Li *et al.*, 2009; Rajabi *et al.*, 2016; Rubio *et al.*, 2018).

The influence of wing flexibility on aerodynamics is not usually disputed, but the details are a matter of debate. Even though the basic aerodynamics of stiff and pliant wings may be the same, publications like Young *et al.* (2009) or Zhao *et al.* (2010) stress the importance of flexibility. There are different ways of describing an objects' flexibility or stiffness. It is possible to use material properties, or explicit or implicit combinations of material properties and properties of the shape of the object. Typical ways of describing stiffness are explained in detail by Vogel (2007), and some will briefly be named here. Perhaps the most widely used material property in this context is Young's modulus of elasticity E. It gives the slope between tensile stress and tensile strain in a material, which in general cannot be assumed to be constant. E is also not necessarily constant within an object, especially for biological samples. One "combined" property measured used quite often is the flexural stiffness or flexural rigidity EI, which is the product of the Young's modulus E and the second moment of area I. I is a property of the cross-section of the object. EI is therefore independent of the length of the object. If material and cross-section are constant throughout the object, the EI will be the same, no matter how long the object is. A value less independent of the length is the spring stiffness k, which gives the ratio between force and deflection. It does not directly allow insight into the geometry or material properties of the object, and consequently does not inherently assume those properties to be constant along the object. All of these metrics have been used in studies on insect wing stiffness before.

The significance of wing flexibility for aerodynamics does not appear to be straightforward. A wing beat frequency below the resonance frequency, which also depends on wing flexibility, may help stabilize flight in dragonflies (Rajabi *et al.*, 2016). Often, studies find that more flexible wings produce more upwards force (Mountcastle and Daniel, 2009; Nakata and Liu, 2012; Mountcastle and Combes, 2013), or better lift-to-power or lift-to-drag ratios (Zheng *et al.*, 2013; Nguyen *et al.*, 2016). Flexible wings may also cost less in terms of energy, depending on the kinematics (Jankauski *et al.*, 2018; Reid *et al.*, 2019).

In contrast to these positive effects, Tanaka *et al.* (2011) found more lift produced by an artificial hoverfly wing when it was stiff and not flexible. The passively achieved negative camber of the flexible wings in their experiments, however, was the opposite of real insect wing camber (which is usually positive). Tobing *et al.* (2017) found that a bumblebee wing model with variable stiffness failed to reach the efficiency of models with uniform stiffness. They proposed that variable stiffness may have other advantages, such as mitigation of consequences from collisions (see also Mountcastle and Combes, 2014, and section 3.2.3), flight stabilisation

(as also proposed by Mistick *et al.*, 2016), or that their modelled stiffness was not realistic enough. They used a Young's modulus E of 110 GPa for their uniformly flexible model, with 11 GPa for the tip of their flexible-tip wing. They calculated the value of 110 GPa from the flexural stiffness EI measured by Combes and Daniel (2003a), and used it for their uniformly flexible model and the proximal part of the flexible-tip model. The distal part of the wing, which was not included in the measurement by Combes and Daniel (2003a), was assumed to have a Young's modulus of 11 GPa. Their estimation of E is based on an assumed rectangular cross-section (used to calculate the second moment of area I) despite the fact that corrugation increases I (mentioned e.g. by Ning *et al.*, 2017). This probably resulted in an overestimate for E. Their value of 110 GPa is considerably higher than the range of Young's modulus of cuticle given by Vincent and Wegst (2004), which spans from approximately 1 kPa for intersegmental membrane to a maximum of approximately 30 GPa for elytra, and for wings provides values around 6 GPa (specific species are not mentioned). These details matter: Truong *et al.* (2020) assumed a Young's modulus of 0.7 MPa for a "highly flexible" bumblebee wing model, and 7 GPa for a "flexible" model (both of which produced less lift but were more efficient than a stiff model), resulting in much more flexible wings compared to Tobing *et al.* (2017). Both studies used somewhat similar CFD bumblebee models, but the relative evaluation of different types of wing models differ between them, likely due to the large discrepancy in Young's modulus.

Similarly, Fu *et al.* (2018) utilized a single wing beat of simplified rectangular wings and determined three general stiffness categories, namely stiff, flexible and very flexible. For stiffness above a certain threshold value, lift and drag coefficients were nearly constant. Flexible wings showed a decrease in drag coefficient but exhibited at most a slight increase in lift coefficient. This was because during accelerations higher lift was produced, while some lift was lost during the uniform motion. For very flexible wings, both lift and drag coefficient dropped, and the LEV was smaller than in the other categories. They found these categories for wings with aspect ratio two and four, but the threshold stiffness values between the categories differed for different aspect ratios. The authors suggest that dorso-ventral stiffness anisotropy would allow insect wings to fall into the flexible category during the downstroke but into the very flexible category during the upstroke. This could explain more lift being generated during the downstroke. Their results fit well to the fact that domesticated flightless silkmoths have more flexible wings than their wild counterparts (Lu *et al.*, 2020).

In general, different species differ in their wing stiffness. For example, Combes and Daniel (2003a) found higher flexural stiffnesses in larger species and Meresman *et al.* (2020) observed differences between two scarab beetle species of similar size but with slightly different capability of controlled landings, and suggest that differences in wing flexibility may correlate with different ecological niches. Therefore, using species-specific wing stiffness when investigating wing flexibility in the context of flight appears to be crucial.

Motivation and methods

As explained above, species-specific knowledge on wing stiffness is important for successful detailed aerodynamic models of flying insects. Flexible wing models have an advantage over models with prescribed deformations (such as for example Le *et al.*, 2013) in that they can be used flexibly for any kinematics and flow, not only those present during the deformation measurement. In order to be able to expand the models used in Engels *et al.* (2020), data on wing stiffness of all three fly species was necessary. Numerous studies investigating wing flexibility and deformation or its influence on flight aerodynamics exist, but most have been done on bigger insects like locusts (e.g Wootton, 1981; Young *et al.*, 2009), dragonflies (e.g Newman and Wootton, 1986; Tong *et al.*, 2015) or moths and butterflies (Steppan, 2000; Moses *et al.*, 2017). Some studies exist on bees (Mountcastle and Combes, 2013; Ning *et al.*, 2017) and bigger dipterans (Combes and Daniel, 2003a; Walker *et al.*, 2009b; Tanaka *et al.*, 2011; Tong *et al.*, 2015; Rubio *et al.*, 2018), including species of *Calliphora* (Combes and Daniel, 2003a; Ganguli *et al.*, 2010; Lehmann *et al.*, 2011). Less well investigated are smaller insects like termites or mosquitoes (Yin *et al.*, 2018). For two of the three species under consideration (*D. melanogaster*, *M. domestica*) no stiffness data seem to have been published. Therefore, measuring wing flexibility in all three species created the opportunity of expanding the number of species for which such data are available and also comparing the results for *C. vomitoria* with previously published data. Due to the fact that spring stiffness k and average flexural rigidity EI are routinely used in the literature, both of them were calculated during the study Wehmann *et al.* (2019) in order to best allow this comparison.

In order to measure physiological stiffness values, particular care was taken to avoid desiccation of the wings. It is known that wings lose moisture once separated from the body and that dry wings are much stiffer. In desiccation experiments on butterfly wings, Steppan (2000) found a sixfold increase in flexural stiffness, while Mengesha *et al.* (2011) observed a doubling in spring stiffness over the course of a day, and the Young's modulus of a dragonfly wing's leading edge increased twenty times when it was dried (Chen *et al.*, 2013). Therefore, wing stiffness was measured in wings attached to living flies in Wehmann *et al.* (2019). Nevertheless, the immobilization of wing and fly may have interfered with the proper function of the wing pulsatory organs (for details on the wing circulatory system, see e.g. Pass *et al.*, 2015; Pass, 2018). This might have resulted in slight changes in wing stiffness, although much reduced compared to detaching the wings or killing the animals.

The experiments were performed by applying point forces to different positions on the wing. The resultant deformation of the entire wing was measured statically. This is a potential weakness of the method because wings may exhibit different stiffness during dynamic loading compared to the static case (Kovalev *et al.*, 2018). This issue is shared with many other studies (e.g. Newman and Wootton, 1986; Combes and Daniel, 2003a; Moses *et al.*, 2017). While deforma-

Table 3.1: Median flexural stiffness of three wing regions in the three species. Data was pooled with respect to individual, dorsal/ventral loading, specific position of load on the wing. $n_{\text{measurement}}$: total number of available data points.

Species	**Region**	$n_{\text{measurement}}$	EI
D. melanogaster	leading edge	22	$5 \times 10^{-11}\,\text{N m}^2$
	wing tip	36	$4 \times 10^{-11}\,\text{N m}^2$
	trailing edge	28	$2 \times 10^{-11}\,\text{N m}^2$
M. domestica	leading edge	30	$5 \times 10^{-9}\,\text{N m}^2$
	wing tip	33	$7 \times 10^{-9}\,\text{N m}^2$
	trailing edge	54	$1 \times 10^{-9}\,\text{N m}^2$
C. vomitoria	leading edge	31	$8 \times 10^{-8}\,\text{N m}^2$
	wing tip	30	$6 \times 10^{-8}\,\text{N m}^2$
	trailing edge	50	$1 \times 10^{-8}\,\text{N m}^2$

tion measurements during flight have been done in several species (e.g. Walker *et al.*, 2009a,b; Koehler *et al.*, 2012), the corresponding forces are usually unknown, preventing calculations of stiffness. The deflection in Wehmann *et al.* (2019) was relatively small (few percent). On the one hand, this allowed the application of simple beam theory to calculate an overall flexural stiffness by estimating a bending line between the fixed wing root and the point of force application. On the other hand, the overall small deflection hindered recognition of possible deviations from such a simple bending line. Such deviations would result from non-uniform stiffness and may give more information on how the wing is structured mechanically, for example by veins or joints. Measuring deformation of the entire wing (similar to measurements by Yin *et al.*, 2018, although their specimens were detached from the bodies) was a way to mitigate this problem, and these data illustrate that wing veins act as structuring elements.

Contextualization of results

Some known general trends in the distribution of wing stiffness were also found in the present study: the fly wings were more flexible at the tip than the base, which is the case for a variety of species (see e.g. Steppan, 2000 for butterflies and Moses *et al.*, 2017 for moths, or Ganguli *et al.*, 2010; Lehmann *et al.*, 2011 for blowflies). It is known that insect wings are generally more flexible in the chordwise than the spanwise direction (Combes and Daniel, 2003a; Ning *et al.*, 2017). While no direct chordwise measurements were performed here, the data show that the trailing edge region possesses low flexural stiffness compared to leading edge region and wing tip area (all measured from wing base), which is in line with lower chordwise stiffness (see table 3.1).

Dorso-ventral anisotropy in stiffness might explain differences in lift production between up- and downstroke (Fu *et al.*, 2018), but this anisotropy cannot be assumed to be a general feature of insect wings. It has been found previously in *Manduca sexta* (Combes and Daniel, 2003b), *Apis mellifera* (Ma *et al.*, 2017) and *Calliphora* sp. (Ganguli *et al.*, 2010). In the present study,

M. domestica wings also exhibited dorso-ventral anisotropy. In other experiments, no such anisotropy could be found in more than a dozen different species, among them *M. sexta* and *Calliphora* sp. (Combes and Daniel, 2003a,b). Similarly, no dorso-ventral anisotropy for *C. vomitoria* and *D. melanogaster* was seen in Wehmann *et al.* (2019). Especially considering that studies on *M. sexta* and *Calliphora* sp. disagree on the presence of dorso-ventral stiffness anisotropy, further investigation into the details of stiffness variation of insect wings is warranted.

The results of stiffness measurements of *C. vomitoria* allows comparisons of the study presented here and previous studies on *Calliphora* sp. Near the wing base, flexural and spring stiffness were found to be $80\,\mathrm{nN^2}$ and $15\,\mathrm{N\,m^{-1}}$, respectively, when applying forces along a line from anterior to posterior wing margin (Lehmann *et al.*, 2011). At a similar distance from the wing base, but with a point force near the posterior margin (point 8 in fig. 9 of Wehmann *et al.*, 2019), the data show much lower median stiffness values of $1.5\,\mathrm{nN^2}$ and $0.1\,\mathrm{N\,m^{-1}}$, respectively. This can be explained by the fact that the application of a point force at the flexible posterior part of the wing – in contrast to that of a line force across the wing – avoids measuring the stiffness of the thick veins near the wing base. Closer to the middle of the wing, measurements at points 6 and 11 in the strongly veined anterior part of the wing yielded values closer to the ones determined via line force application, with a flexural stiffness of $40\,\mathrm{nN^2}$ and $90\,\mathrm{nN^2}$ for the point forces (Wehmann *et al.*, 2019) and $120\,\mathrm{nN^2}$ for the line force (Lehmann *et al.*, 2011). The spring stiffness for the point forces ($2.5\,\mathrm{N\,m^{-1}}$, Wehmann *et al.*, 2019) is approximately half of that of the line forces ($4.5\,\mathrm{N\,m^{-1}}$, Ganguli *et al.*, 2010; Lehmann *et al.*, 2011). In a more distal part of the wing, point force application (points 5, 10) resulted in measured median spring stiffness of $0.4\,\mathrm{N\,m^{-1}}$ (Wehmann *et al.*, 2019), compared to $1.5\,\mathrm{N\,m^{-1}}$ and $2.7\,\mathrm{N\,m^{-1}}$ for line forces (Ganguli *et al.*, 2010; Lehmann *et al.*, 2011). The flexural stiffness determined by line force application is approximately $100\,\mathrm{nN^2}$ (Lehmann *et al.*, 2011). The flexural stiffness at points 5 and 10 is $20\,\mathrm{nN^2}$ and $50\,\mathrm{nN^2}$, respectively (Wehmann *et al.*, 2019). Another study using point force application at a similar distance to the wing base and near the leading edge found a flexural stiffness of $1.3\,\mathrm{\mu N^2}$ (Combes and Daniel, 2003a), a value vastly different from the others. While part of the higher stiffness may be explained by the stronger venation closer to the leading edge than points 5 and 10, the value is also much larger than that found at point 1, directly at the leading edge at a slightly more proximal position ($100\,\mathrm{nN^2}$, Wehmann *et al.*, 2019). The likely main cause of the discrepancy is increased stiffness due to desiccation in the data from Combes and Daniel (2003a), although they took care to complete the measurements within an hour after removing the wing from the fly, a time span they had selected empirically to avoid changes in stiffness. An hour is also approximately the time span for completing measurements at all 11 locations on one wing, excluding the time needed to prepare the fly, in Wehmann *et al.* (2019). Lehmann *et al.* (2011) sealed the cut at the wing base within two minutes and completed all measurements within half an hour. Despite the application of line forces instead of point forces, their values are much closer to the ones from Wehmann *et al.*

(2019). What part of the difference between the data in Wehmann *et al.* (2019) on the one hand and Ganguli *et al.* (2010) and Lehmann *et al.* (2011) on the other is due to differences in force application, moisture levels inside the wing or fixation at the wing base is uncertain. It can be tentatively assumed that the values in Combes and Daniel (2003a) correspond less well to a physiological wing state than those in Ganguli *et al.* (2010), Lehmann *et al.* (2011), and Wehmann *et al.* (2019).

There is a large body of data on wing stiffness in different insect species, but the above paragraph shows that direct comparison of studies can be difficult due to different experimental approaches and wing states. The experiments for Wehmann *et al.* (2019) were designed to minimise changes in the wing compared to its natural state. This was especially important because apart from increasing the number of species with known wing flexibility, one aim of the study was to collect data as a basis for wing models with realistic flexibility. It appears that the goals of the study were achieved. Consequently, the main limitation of the data is due to the experimental approach of performing static measurements of deformation using small deflections. Despite this, the data have successfully been incorporated into a numerical wing model of *C. vomitoria* by Truong *et al.* (2022).

3.2.3 Wing wear

The study discussed here is "Flight activity and age cause wing damage in house flies" (Wehmann *et al.*, 2022, included in this thesis, see p. III).

Insects are manoeuvrable fliers (see e.g. Lehmann and Dickinson, 1998; Wang *et al.*, 2003; Bergou *et al.*, 2010; Chakraborty *et al.*, 2015) and when taking off from a sitting position, they can produce forces equivalent to up to five times their body weight (Marden, 1987). The main vortical structure for lift production is the leading edge vortex (see section 2.4), and the trailing edge of the wing is often flexible (see section 3.2.2), although stiffening the trailing edge can increase lift and efficiency (shown for a robot by Tanaka *et al.*, 2015). What is the purpose of this seemingly unnecessary wing area? Safety margins may be an explanation. The idea that "surplus" area may help keep wings and fins functional even when damaged has been proposed for fish, bats and insects (Davis, 1968; Webb, 1973; Kingsolver, 1999; Sinclair *et al.*, 2011; Vance and Roberts, 2014). Insects accumulate wing damage and these injuries cannot heal in adult insects (see e.g. Kříženecký, 1914; Rajabi *et al.*, 2020), so this may be especially relevant for them. Therefore, the last study presented here is concerned with wing wear in the medium sized *M. domestica*.

Reasons for and consequences of wing damage

Reasons for wing damage include collisions with objects (Burkhard *et al.*, 2002; Foster and Cartar, 2011; Rhainds and Brodersen, 2012), or more generally locomotor activity (mentioned

e.g. in Hayes *et al.*, 1998) but also interactions with other animals, such as mating behaviour (for flies, see e.g. Ragland and Sohal, 1973; Burkhard *et al.*, 2002). This includes fights between males (Dawson's burrowing bee: Alcock, 1996; butterflies: Pinheiro, 1990), oviposition (dragonflies: Rajabi *et al.*, 2020) and building and defending nest tunnels (suggested for solitary bees by O'Neill *et al.*, 2015). Intraspecific aggression (Nalepa, 2012) and competition (O'Neill *et al.*, 2015) have also been discussed as reasons for wing damage in hymenopterans, and initially asymmetric wings gain additional damage faster than more symmetric ones in honeybees (Higginson and Barnard, 2004). Allsopp (1985) also proposed for flies that the weather, especially the humidity, might influence the rate of damage acquisition because dry wings become more brittle (see also reviews on the wing circulatory system, e.g. Pass *et al.*, 2015). Damage levels may vary according to sex (housefly: Ragland and Sohal, 1973; hymenopterans: Mueller and Wolf-Mueller, 1993; Lee *et al.*, 2006; lepidopterans: Rhainds and Brodersen, 2012; Kiritani *et al.*, 2013) or sex ratio of a group (shown for a moth by Rhainds and Brodersen, 2012), though not in all species (e.g. the dragonfly *Sympetrum vulgatum*; Rajabi *et al.*, 2017). To sum up, one important direct cause of wing damage are collisions. How many collisions a wing encounters depends on both the environment and the behaviour of the insect.

Wing damage directly affects the aerodynamics of the wing, but indirect effects such as the loss of sensory information or decoupling of wing and haltere motion and the resultant challenges for flight control may also be relevant (see section 2.3.4). Wing damage usually leads to active or passive changes in wing kinematics (e.g. the relative timing of left and right wings in *M. sexta*; Fernández *et al.*, 2012) and adjustments of body posture. Active changes of the kinematics are similar to the ones used for manoeuvring (reviewed in Taylor, 2001). Among the parameters that potentially change as a result of damage are wing beat frequency (Diptera, Hymenoptera, Lepidoptera, Odonata: Sotavalta, 1952a; Danzer, 1956; Kingsolver, 1999; Jantzen and Eisner, 2008; Buchwald and Dudley, 2010; Fernández *et al.*, 2012; Vance and Roberts, 2014; Roberts and Cartar, 2015; Kassner *et al.*, 2016; Muijres *et al.*, 2017; Kihlström *et al.*, 2021), wing beat amplitude (Diptera, Hymenoptera, Odonata: Fernández *et al.*, 2012; Vance and Roberts, 2014; Kassner *et al.*, 2016; Fernández *et al.*, 2017; Muijres *et al.*, 2017; Lyu *et al.*, 2020; Kihlström *et al.*, 2021, though not always, as in the bumblebees in Hedenström *et al.*, 2001), as well as wing or stroke plane angles and exact timing of the motion (Diptera, Lepidoptera, Odonata: Fernández *et al.*, 2012; Kassner *et al.*, 2016; Muijres *et al.*, 2017; Lyu *et al.*, 2020). Body posture alterations have been shown for example in *Drosophila hydei* (Muijres *et al.*, 2017) and *Macroglossum stellatarum* (Kihlström *et al.*, 2021). Apart from kinematics and body posture, wing damage may also affect energetics. Metabolic power increased in *M. sexta* with asymmetrically clipped wings (but not symmetrically clipped wings; Fernández *et al.*, 2017), but butterflies with about 20 % reduction in total wing area were not observed to more frequently drink nectar (Kingsolver, 1999), and bumblebees showed no change in CO_2 production upon removal of 10 % of forewing area (Hedenström *et al.*, 2001). It also appears as

if more wing-damaged solitary carpenter bees (*Ceratina calcarata*), possibly due to energetic reasons, build nests later and have less brood (both in terms of number and weight; Rehan and Richards, 2010), indicating wing damage may influence fitness. This is also true for species using their wings for courtship, such as *Drosophila* (Davis *et al.*, 2018), where the effect is probably much more direct than for the bees. In conclusion, changes in at least some kinematic parameters due to wing damage are to be expected, and it stands to reason that, at least for significant damage, an animal's energy balance and potentially its fitness will be affected.

Most wing damage observed in animals in a natural habitat is less than 10 %, but specimens with 25 % or even 75 % damage have been observed (bumblebees, dragonflies: Cartar, 1992; Rajabi *et al.*, 2017). 75 % to 80 % of *S. vulgatum* individuals have at least slightly damaged wings (Rajabi *et al.*, 2017), but only few female *C. calcarata* showed considerable damage (Rehan and Richards, 2010). Severe wing damage can result in a total loss of flight ability. This happens at approximately 20 % to 30 % area loss in flies (Ragland and Sohal, 1973), bumblebees (Haas and Cartar, 2008) and locusts (Fischer and Kutsch, 2000). Not only the amount wing damage but also its asymmetry are relevant. Haas and Cartar (2008) found in bumblebees a decrease in flight speed for strong symmetric damage, but speed increased for asymmetric damage, and only individuals with strong asymmetric wing damage appeared to change the way they fly compared to intact ones. Overall, damage can be compensated surprisingly well, as adjustments in wing kinematics and body posture appear to mitigate the effects of wing damage, though not always completely. Overall, the amount of damage, levels of damage asymmetry, and how demanding any tests are, all influence whether or not effects of wing damage are found (Haas and Cartar, 2008; Vance and Roberts, 2014; Kassner *et al.*, 2016; Fernández *et al.*, 2017). Detrimental consequences of wing damage on aerodynamics observed in studies using models could in part be explained by the kinematics used being optimized for intact wings (suggested for hummingbirds by Chai, 1997 and for moth models by Kihlström *et al.*, 2021), highlighting the importance of behavioural changes to compensate for wing damage.

The aforementioned compensatory mechanisms may also be less effective when the insects are old (investigated in bees by Vance, 2009). Insects experience senescence of the entire flight apparatus (flies, bees: Rockstein and Bhatnagar, 1965; Yan and Sohal, 2000; Higginson and Gilbert, 2004; Miller *et al.*, 2008; Lane *et al.*, 2014), locomotor activity and endurance are reduced (flies: Rockstein and Bhatnagar, 1966; Grotewiel *et al.*, 2005), and even wing beat frequencies change (flies, locusts, bees: Rockstein and Bhatnagar, 1966; Fischer and Kutsch, 2000; Vance, 2009; Lane *et al.*, 2014; Eichorn *et al.*, 2017). Additionally, ageing wings slowly desiccate and become more brittle (e.g. Arnold, 1964; Pass *et al.*, 2015; Salcedo and Socha, 2020), possibly making them more susceptible to wing damage. Flies kept in captivity can grow older than their wild counterparts, possibly due to lack of predators and competition as well as high quality resources for little effort (Pitts and Wall, 2004). In general, activity seems to reduce lifespan. Activity levels are likely the reason why population density and sex ratio in cages as

well as temperature change life expectancy (*M. domestica*, see e.g. Ragland and Sohal, 1973, 1975; Sohal and Buchan, 1981), even though how activity is influenced by these factors cannot always easily be predicted. While flight activity is not always found to reduce lifespan (reviewed in Guo *et al.*, 2020), there appears to be, at least in some species, an influence of locomotor activity on the rate of physiological senescence (flies, bees: Rockstein and Bhatnagar, 1965; Ragland and Sohal, 1973, 1975; Sohal and Buchan, 1981; Cartar, 1992; Yan and Sohal, 2000). This is sometimes expressed as a "lifetime flight activity budget", described for honeybees either in terms of total flight distance (foragers appear to die after about 800 km, suggested by Neukirch, 1982, to be due to limits of the glycogen metabolism) or total number of wing beats the flight motor system can perform (Higginson and Gilbert, 2004). The latter, through increased flapping frequency, may explain why bees with damaged wings sometimes seem to die earlier (Higginson and Gilbert, 2004; Higginson *et al.*, 2011). Alternatively, compensating for wing damage might result in faster physiological senescence (proposed for bumblebees by Cartar, 1992). To sum up, activity, general senescence and wing damage accumulation appear to possibly influence each other. Similarly, general senescence and wing damage as well as interactions between the two factors are detrimental to flight ability and longevity.

Motivation and methods

It is known that wing damage is caused by collisions, and therefore probably indirectly by flight activity, and that its aerodynamic consequences intertwine with those of general senescence (see above). This, in turn, means that any influence of wing damage on fitness likely also strongly depends on the particular life history of an individual animal. Wing damage influences flight kinematics and possibly energetics (see above), therefore researchers should take into account wing state when using insects in flight experiments, and they should be aware of kinematic changes when modelling the aerodynamics of damaged wings. If its age is known, levels of damage may also indicate how active an animal has been. Most studies to date that have implied that collisions are a prominent cause for wing damage did not include detailed measures of both wing damage and activity over time (e.g. Allsopp, 1985; Hayes *et al.*, 1998; Foster and Cartar, 2011; Rhainds and Brodersen, 2012). The study done in Wehmann *et al.* (2022) was performed with the aim of changing this, by investigating correlations of age, wing damage, and activity or flight ability by recording these parameters for the entire lives of individual *M. domestica*.

Activity was measured using audio recordings. This approach allowed automated recognition of the buzzing sound of flapping fly wings. To ensure that flies were not far from the microphone, small containers were used, despite the fact that they limited the freedom of the flies to move. In order to encourage the animals to be active, groups of four flies were placed inside each container, which also increased the total number of individuals in the experiment. However, in this approach within each group only the average activity per individual could be determined, which is a limitation of the study. All activity of the flies was supposed to be recor-

ded as completely as possible, therefore flight ability was tested in a separate experiment. This experiment included a group of flies living in a larger flight cage without audio recordings. This allowed comparison of wing damage progression under two rather different conditions, as well as regular testing of the flies' ability to fly from the centre of the cage to one of the walls.

Wing state was regularly recorded by taking photos of the flies and manually tracing the wing outline (using Fiji; Schindelin *et al.*, 2012). There are different ways to qualitatively or quantitatively measure wing damage. A few examples will be mentioned here. Sometimes wings are grouped into typically five to seven groups according to the level of damage, counted as number and/or severity of injuries according to different definitions (Cartar, 1992; Mueller and Wolf-Mueller, 1993; Alcock, 1996; Rehan and Richards, 2010; O'Neill *et al.*, 2015). Generally speaking, the level of measurement of this type of damage quantification is ordinal, although specific implementations may be using ratio scales. For spruce worms, the relative wing area covered in scales was used by Rhainds and Brodersen (2012); Rhainds (2015). In most cases, however, area loss is used as a measure of damage, sometimes in absolute terms (Niitepõld and Boggs, 2015) but usually as a relative area. The reference value is either the total area of all wings (Kingsolver, 1999; Fischer and Kutsch, 2000; Haas and Cartar, 2008; Buchwald and Dudley, 2010; Salcedo *et al.*, 2014) or the area of one wing (Ragland and Sohal, 1973; Fischer and Kutsch, 2000; Haas and Cartar, 2008; Combes *et al.*, 2010; Higginson *et al.*, 2011; Fernández *et al.*, 2012; Salcedo *et al.*, 2014; Mountcastle *et al.*, 2016; Lyu *et al.*, 2020). In some cases, the reference area excludes the proximal part of the wing (Foster and Cartar, 2010, 2011; Nalepa, 2012; Mountcastle and Combes, 2014). This is often reasonable because wings tend to first lose area in the outer part or at the trailing edge (Cartar, 1992; Rajabi *et al.*, 2017). As a measure of wing damage, Wehmann *et al.* (2022) therefore used relative wing area remaining, with the reference area excluding the most proximal part of the wing, which was hard to see without potentially injuring the animals.

In the interest of investigating wing damage in (relatively) freely behaving animals, few factors apart from group size (only in the container experiment) and light cycles (more strictly in the container experiments) were controlled. Due to the resultant strong correlation between age and activity, the study does not offer a way to confidently disentangle the contributions of age, activity and their interaction to wing damage. The same is true for the contributions of age, wing damage and their interaction to loss of flight ability. One of the strengths of the study in Wehmann *et al.* (2022) is that wing damage, age and time spent active or flight ability were all recorded at regular intervals for individual flies for their entire lives.

Contextualization of results

The fact that wing damage in insects accumulates over time has been used to investigate the option of age grading insects via wing state (e.g. Allsopp, 1985; Hayes and Wall, 1999; Irvin

and Hoddle, 2009; Hargrove, 2020). This approach to determining the age of an insect has limitations because sudden increases in wing damage can occur, for example because of predator attacks (Lepidoptera, Hymenoptera, Diptera: Benson, 1972; Mueller and Wolf-Mueller, 1993; Burkhard *et al.*, 2002), but more generally because wing damage depends on behaviour (reviewed in Hayes and Wall, 1999) and habitat (parasitoid wasp: Lee *et al.*, 2006). Wehmann *et al.* (2022) also found that wing damage correlated with both age and activity and their respective influences could not be disentangled. Additionally, there was strong interindividual variability even for flies kept under the same conditions.

The progression of wing damage in the flies of Wehmann *et al.* (2022) followed a logistic curve. This fits with data from previous studies finding increasing wing damage rates over time for bees (Higginson and Barnard, 2004; Foster and Cartar, 2011), corresponding to the first part of a logistic curve. The study by Mountcastle and Combes (2014) observed a decrease of wing damage rates over time during artificial collisions of yellowjacket and bumblebee wings with an acrylic plate covered by a leaf. This may correspond to the second part of a logistic curve. The first part (increasing damage rates) may not be visible because of the nature of the experiment, which included harsh damage application (constant collisions with a rigid object at 216 Hz) and a pre-determined maximum wing damage (fixed distance of the animal to the wall). It is also possible that this experimental setup results in a different wing damage progression than experiments in freely behaving animals. For the solitary bee *Anthidium manicatum*, Mueller and Wolf-Mueller (1993) fit a cubic polynomial model to their data on wing damage progression in individuals from a wild population, capturing an initial high rate of damage accumulation which then decreased over time. This damage progression at first glance appears different from the one observed by Wehmann *et al.* (2022), because it is also missing the first part of the logistic curve, but the results cannot be compared due to the difference in wing damage quantification. While Wehmann *et al.* (2022) used relative remaining wing area, Mueller and Wolf-Mueller (1993) placed each wing into one of seven discrete classes. The classification did not depend linearly on the remaining wing area, and focused on the distal part of the forewing. The latter point hints that damage begins at the wing tips also in this species, which is in line with the observations of Wehmann *et al.* (2022) as well as results from *Bombus melanopygus* (Cartar, 1992) and *S. vulgatum* (Rajabi *et al.*, 2017).

Wehmann *et al.* (2022) found that flight ability decreases with increasing age, wing damage levels, and wing damage asymmetry, which fits with the existing literature (see section on the reasons of wing damage above as well as Miller *et al.*, 2008; Fernández *et al.*, 2017). Flies in the present study seemed to be unable to fly once they lost 10 % to 34 % wing area (similar to values found previously by Ragland and Sohal, 1973; Fischer and Kutsch, 2000; Haas and Cartar, 2008), or for left-right area asymmetries of greater than 25 %. The test for flight ability was rather simple and offered no further insight into the aerodynamic consequences of wing damage and possible kinematic compensations performed by the fly. However, the detailed observations

of the damage patterns that occurred could in future be used as a basis to perform more sophisticated flight experiments with artificial wing damage modelled after the one observed here. This type of behavioural experiment, or CFD simulations based on the results obtained in this project may allow investigations of the influence of naturally occurring wing damage without confounding factors like previous activity or senescence of the flight motor.

3.3 Conclusion

The results of this thesis provide insights into three-dimensional corrugation, flexibility and wear of the wings of three fly species in the context of flight. Micro-computed tomography and optical profilometry allowed the creation of detailed wing models including corrugation and camber (Engels *et al.*, 2020). The results of fluid-dynamic simulations of these models support the idea that camber is an important factor for wing aerodynamics, while corrugation does not seem to have direct aerodynamic ramifications. Static deformation measurements of fly wings under point loads highlighted the mechanical complexity of insect wings (Wehmann *et al.*, 2019). The results have been successfully used as a basis for a flexible wing model by (Truong *et al.*, 2022). This type of model may be used for further computational fluid dynamics simulations of insect flight, including fluid-structure interactions and therefore interactive conformational changes of the wing. The last part of the work focused on damaged wings and produced a detailed account of damage patterns and progression according to age and activity in houseflies, as well as of flight ability depending on age and wing damage (Wehmann *et al.*, 2022). While the original questions on what causes wing damage and loss of flight ability could not be answered as clearly as initially hoped, the information gathered on naturally occurring wing damage in this study can be used as a template for artificial damage in follow-up studies on the aerodynamics of damaged wings. Overall, this thesis adds to what we know about insect wings and offers a detailed basis for realistic flexible aerodynamic wing models of the three differently-sized fly species *Drosophila melanogaster*, *Musca domestica*, and *Calliphora vomitoria*. It may therefore serve as a valuable basis for further research on the ecology and biophysics of insect flight.

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Appendix A

Manuscripts

A.1 Manuscript 1: Corrugation and aerodynamics

The manuscript has been published as:

Engels, T.*, Wehmann, H.-N.*, Lehmann, F.-O. (2020): Three-dimensional wing structure attenuates aerodynamic efficiency in flapping fly wings. *Journal of The Royal Society Interface* 17: 20190804. https://doi.org/10.1098/rsif.2019.0804
*These authors equally contributed to this work.

A.2 Manuscript 2: Wing flexibility

The manuscript has been published as:

Wehmann, H.-N., Heepe, L., Gorb, S. N., Engels, T., Lehmann, F.-O. (2019): Local deformation and stiffness distribution in fly wings. *Biology Open* 8: bio038299. https://doi.org/10.1242/bio.038299

A.3 Manuscript 3: Wing damage progression

The manuscript has been published as:

Wehmann, H.-N., Engels, T., Lehmann, F.-O. (2022): Flight activity and age cause wing damage in house flies. *Journal of Experimental Biology* 225: jeb242872. https://doi.org/10.1242/jeb.242872

Appendix B

Declarations and acknowledgements

B.1 Erklärung über den Eigenanteil

Alle angeführten Veröffentlichungen sind unter meiner Erstautorenschaft oder unter geteilter Erstautorenschaft entstanden. Alle Autoren haben an der Manuskriptentstehung mitgewirkt.

Three-dimensional wing structure attenuates aerodynamic efficiency in flapping fly wings.
(Manuskript siehe Seite I)

- geteilte Erstautorenschaft mit Thomas Engels (50 %)
- Mitwirkung am Studiendesign (25 %)
- Datenerhebung (95 %)
  - Fixierung der Fliegen unter Anleitung
  - Organisation der Computertomographie-Scans der Fliegen (Bedienung des Tomographen durch Dr. Christian Wirkner und Stephan Scholz)
  - Manuelle Segmentierung der Scans und Export als Bildstapel
- CFD (5 %)
  - Bereitstellung der Flügeloberflächenprofilscans (Datensätze aus Manuskript 2, siehe unten)
  - Zuarbeit für die 3D-Modelle der Flügel (Modellerstellung: Thomas Engels)
- Manuskript
  - Verfassen des ersten Entwurfs gemeinsam mit Thomas Engels (50 %); meine Schwerpunkte waren:
    * Literaturrecherche zum Vorkommen und zum Einfluss der Flügelfältelung
    * Methodenteil: Computertomographie
    * Abbildungensentwürfe: Computertomographie, Kinematik und Winkel
    * Interpretation und Einordnung der Ergebnisse
  - Überarbeitung des Manuskripts gemeinsam mit den Ko-Autoren (33 %)

Local deformation and stiffness distribution in fly wings. (Manuskript siehe Seite II)

- Erstautorenschaft
- Mitwirkung am Studiendesign (33 %)
- Methodenentwicklung (60 %)
- Praktische Durchführung (100 %)
  - Experimente zur Austrocknung von Flügeln
  - Bezug des Kraftsensors von der CiS Forschungsinstitut für Mikrosensorik GmbH
  - Präparation und Durchführung der Oberflächenscans (in Professor Gorbs Labor an der Universität Kiel, nach Einweisung durch Dr. Lars Heepe)
  - Berechnung der Kräfte, Verformungen und Steifigkeiten
  - Auswertung der Daten
- Manuskript
  - Verfassen des ersten Entwurfs (100 %)
  - Überarbeitung des Manuskripts gemeinsam mit den Ko-Autoren (33 %)

Flight activity and age causes wing damage in house flies. (Manuskript siehe Seite III)

- Erstautorenschaft
- Mitwirkung am Studiendesign (50 %)
- Methodenentwicklung (60 %)
- Praktische Durchführung (75 %)
  - Fangen von Fliegen (mit Hilfe von Annegret Mahrwald, Birgit Wobith, Uta Effmert, Josephine Dieckmann)
  - Durchführen der Experimente zur Flugaktivität und Fliegenfotos (zusammen mit Evan Grant, Bärbel Redlich-Witt, Birgit Wobith)
  - Durchführen der Experimente zur Flugfähigkeit und Fliegenfotos
  - Durchführen der Kontrollexperimente zur Fliegenaktivität gemeinsam mit Thomas Engels
  - Manuelle Erstellung der Flügelumrisse und Setzen der Koordinaten
  - Auswertung der Videos des Kontrollexperiments (zusammen mit Thomas Engels)
  - Datenverarbeitung, -auswertung, und -analyse
    - Bildbearbeitung
    - Berechnen von Flügelflächen und -momenten
    - Bestimmung von Alter und kumulierter Aktivität
    - statistische Auswertung (mit Hilfe von Ratschlägen von PD Dr. Wolf Hanke, Prof. Dr. Alexander Meister, Prof. Dr. Kai Schneider, Dr. Sixin Zhang, einem anonymen Reviewer)
- Manuskript
  - Verfassen des ersten Entwurfs (100 %)
  - Überarbeitung des Manuskripts gemeinsam mit den Ko-Autoren (33 %)

B.2 Acknowledgements

I first and foremost wish to acknowledge Prof. Dr. Fritz-Olaf Lehmann for supervising me and providing me with the chance to work on this project. He also helped me out in the lab with getting me started on LabView[1] and PI MikroMove[2], as well as building the sound recording setup for the wing damage experiments. He was the one to fund my work (project position, stipend).

My research was part of project AIFIT (DFG grant number LE905/17-1) and was for the most part funded as such. My fellow project members provided much discussion and collaboration, especially Thomas Engels, Truong Dinh Hung and Fritz-Olaf Lehmann. Marie Farge, Kai Schneider and Jörn Sesterhenn were also always interested in hearing about the biological side of things and sharing their perspectives.

None of the housefly experiments would have happened if I had not initially been given flies by Marion Lindner of the German Umweltbundesamt.

Dr. Christian Wirkner and Stephan Scholz allowed me access to the µCT belonging to the Richter lab at the University or Rostock. They helped with the sample preparation for the scans and operated the equipment. The computational fluid dynamics simulations of flat and corrugated fly wings (and the final creation of the wing models used) were done by Thomas Engels.

As part of the wing stiffness project I was allowed to visit Prof. Dr. Stanislav Gorb's lab in Kiel, where Dr. Lars Heepe showed me the use of their optical profilometer. Furthermore, the experiments would have been less successful without the kindness of various lab members, especially Nienke Bijma, Shuto Ito, Dr. Hamed Rajabi, Dr. Clemens Schaber, Angela Veenendaal and Joachim Oesert. For measuring the wing stiffness, I used a small force sensor provided by CiS Forschungsinstitut für Mikrosensorik GmbH[3].

The student intern Evan Grant (funded by DAAD RISE 2018) initially helped set up and run the activity experiment as part of the wing damage project and created the first version of the sound analysis algorithm, which was later improved and re-implemented by Thomas Engels.

I had help with data acquisition in the wing damage project from Evan Grant, Bärbel Redlich-Witt and Birgit Wobith (fly photos and sound recordings) as well as Annegret Mahrwald, Birgit Wobith, Josephine Dieckmann and Uta Effmert (who helped catch wild flies).

Especially when analysing the wing damage data, I had helpful discussions on data analysis and statistics with Prof. Dr. Alexander Meister, Dr. Sixin Zhang, Prof. Dr. Kai Schneider and

[1] https://www.ni.com/de-de/shop/labview.html
[2] https://www.physikinstrumente.de
[3] https://www.cismst.de

PD Dr. Wolf Hanke. Furthermore, I want to acknowledge that I used the free software R (R Core Team, 2020) with RStudio[4] for most of my data analysis (single packages are referenced in the manuscripts), and received many helpful hints by browsing stackoverflow.com, r-bloggers.com and similar sites. Additionally, I used Fiji (Schindelin *et al.*, 2012), Gimp[5], Icy (de Chaumont *et al.*, 2012), Inkscape[6], LibreOffice[7], processing[8], Python[9] and other free software.

Over the years I received help with fly keeping and anything lab-related from Annegret Mahrwald, Bärbel Redlich-Witt and Birgit Wobith. For everything to do with administration, Maren Bagrowski was of invaluable assistance. I also never lacked input and help from my fellow lab members, foremost among them Thomas Engels, Ralf Bastrop and Taufia Hussain.

During the work for this thesis, I made use of https://www.connectedpapers.com to find pertinent publications. I also used Google Scholar[10], Web of Knowledge[11] and the library of the University of Rostock. My reference manager was JabRef[12]. The thesis was typeset using LaTeX[13] and TeXStudio[14]. Parts of it have been read by a handful of people who provided constructive criticism. Finally, my friends and family have kindly kept encouraging me whenever I needed it, for which I am grateful.

[4] https://www.rstudio.com/
[5] https://www.gimp.org/
[6] https://inkscape.org/de/
[7] https://www.libreoffice.org/
[8] https://processing.org/
[9] https://www.python.org/
[10] https://scholar.google.com/
[11] https://webofknowledge.com/
[12] https://www.jabref.org/
[13] https://www.latex-project.org
[14] https://www.texstudio.org/