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The Structure and Function of the Dung Beetle Wind Compass

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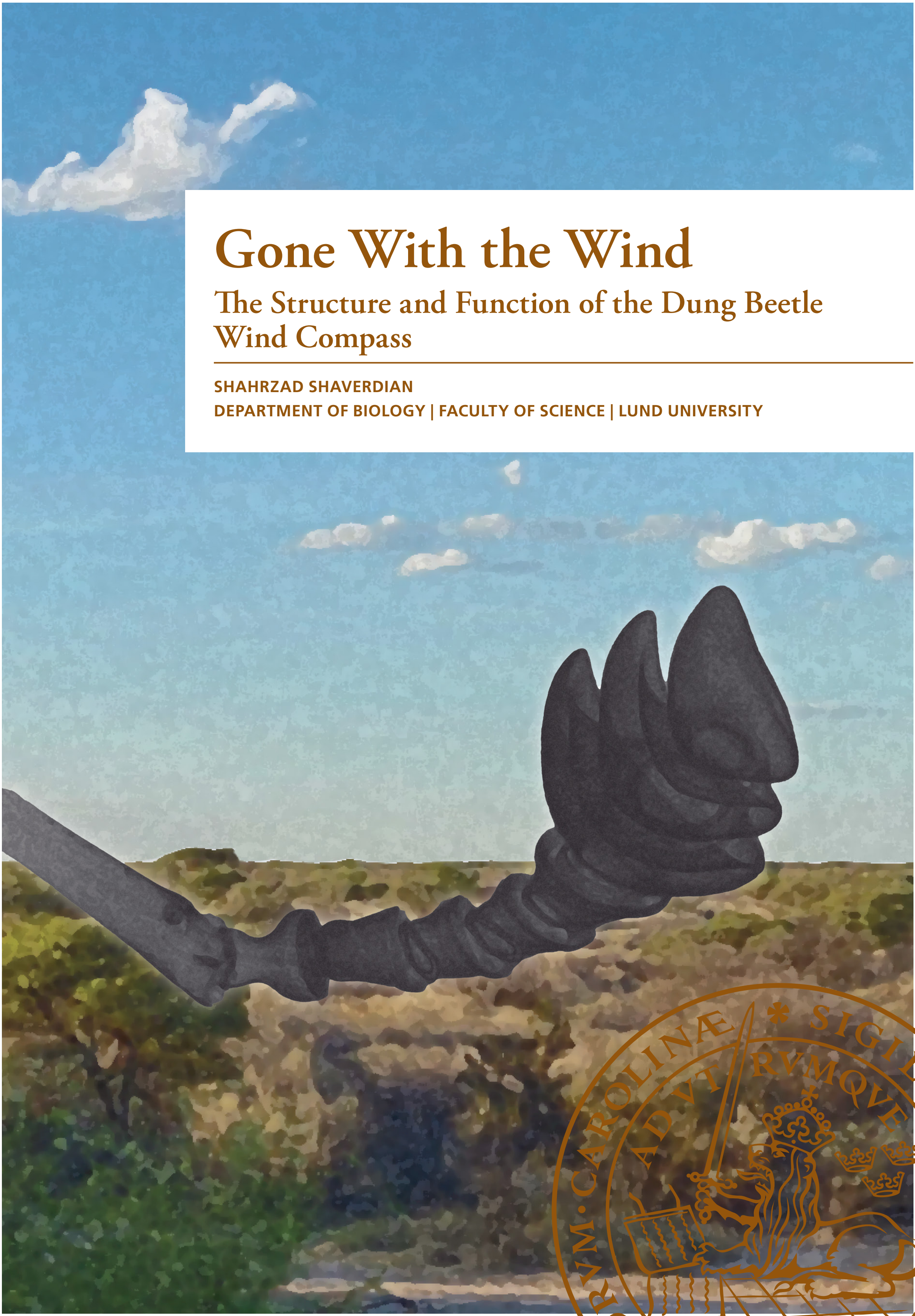

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Shahrzad Shaverdian

LUND
UNIVERSITY

DOCTORAL DISSERTATION

Doctoral dissertation for the degree of Doctor of Philosophy (PhD) at the Faculty of Science at Lund University to be publicly defended on 23 of May at 13.00 in the Blue Hall, Ecology Building, Department of Biology, Sölvegatan 37, Lund, Sweden

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Abstract: Prevailing winds carry seafarers across oceans and guide insects on their journeys. The South African, ball-rolling dung beetle *Kheper lamarcki* depends on a range of compass cues, including the azimuthal position of the sun and the directed airflow of *wind* to steer its plotted course. In this thesis, I set out to further our understanding for how these insects benefit from the directional component of the wind, and how this information is combined with the directional information from their solar compass. In ***Paper I***, I verify that the antennae are the sensors that support wind-guided straight-line orientation in the beetles. I further demonstrate that even though a single antenna can support wind-guided straight-line orientation, the wind compass benefits from the pair. In ***Paper II*** I proceed to characterise the detailed morphology of the wind sensor itself – the antennal Johnston's organ. I find that the beetle's Johnston's organ shares the characteristic morphology found among other wind-orientating insects. In ***Paper III***, me and my colleagues turn our attention to how dung beetles integrate the directional information given by multiple cues and found that the integration strategy at play is a weighted vector summation, in which the relative influence of each cue on the combined directional signal is given by its relative weight. In ***Paper IV***, we expand on this study and propose that the parameter that determines the weight given to each cue is likely its contrast. Taken together, the work presented in this thesis highlights how the dynamic and adaptable insect compass system can guide the animal on its journey, despite the challenges it may face.

Key words: Wind-guided straight-line orientation, Antennae, Johnston's organ, Cue integration

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They turn to sun, to sky so wide,
to daylight’s arc as faithful guide.
With every step, they track the light,
a compass cast in plainest sight.
But when the sunlight slips away,
or clouds drift in to blur the rays,
they do not falter, stall, or stray,
the ***wind*** *will rise and show the way.*

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Scientific Papers

I. **Shaverdian, S.**, and Dacke, M., One antenna can support wind-guided straight-line orientation in the dung beetle *Kheper lamarcki*. (*Manuscript*).

II. **Shaverdian, S.**, Rasmussen, C., and Dacke, M., Ultrastructure of the dung beetle Johnston's organ. (*Manuscript in revision*).

III. **Shaverdian, S.**, Dirlik, E., Mitchell, R., Tocco, C., Webb, B., Dacke, M. (2022). Weighted cue integration for straight-line orientation. iScience 25, 105207. https://doi.org/10.1016/j.isci.2022.105207

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

Author Contributions

I. **S.S.** and M.D. designed experiments. **S.S.** conducted the experiments. **S.S.** and M.D. analysed the data. **S.S.** drafted the manuscript. **S.S.** and M.D. revised the manuscript.

II. **S.S.** and C.R. prepared samples for electron microscopy and collected morphological data. **S.S.** analysed the data. **S.S.** drafted the manuscript. **S.S.** C.R. and M.D. revised the manuscript.

III. **S.S.**, E.D. and C.T. collected and transported beetles. **S.S.**, E.D. and M.D. designed behavioural experiments. **S.S.** and E.D. conducted behavioural experiments, **S.S.** and E.D. analysed behavioural data. R.M. did all of the modelling. **S.S.**, E.D. and R.M drafted the manuscript. **S.S.**, E.D. R.M, C.T., B.W. and M.D. revised the manuscript. First-authorship is shared between **S.S.** E.D. and R.M.

IV. **S.S.** and M.D. designed behavioural experiments. **S.S.** conducted behavioural experiments. **S.S.** and M.D. analysed behavioural data. R.M. did all of the modelling. **S.S.** drafted the parts of the manuscript pertaining to behaviour. R.M, **S.S.** M.D. and B.W. revised the manuscript.

Popular summary

As the scorching sun radiates down on the South African savannah, the air is irregularly heated. This results in pressure gradients that drive the movement of air from areas of high pressure to areas of low pressure. This is what you and I, or a tiny insect, experience as ***wind***. As the wind flows forward across the savannah, it can capture the scent of a fresh dung pile, may eventually cross paths with the foraging, ball-rolling dung beetle *Kheper lamarcki* and guide the beetle to its feast. However, when the beetle arrives at the dung pile, it does so in the company of hundreds of other individuals. To enjoy its meal, the beetle breaks off a piece of dung, sculpts it into a ball and, after fending off a few opportunists, commences its journey to safety – backwards, with its head near the ground and with the ball between its hind legs. The beetle escapes along a remarkably straight path away from the dung pile, effectively maximising the distance between itself and the chaos at the pile with each backwards step that it takes. To maintain their *straight-line orientation*, beetles, much like many other orientating insects, integrate directional information into their *internal compass*, or *head direction network*. The Stimuli that support this compass include sun, the pattern of polarised light formed around the sun, the light intensity gradient across the sky, or prevailing wind. In this thesis, I dive into the structure and function of a *wind compass*, using *K. lamarcki* as my model organism.

The story begins in ***Paper I***, where I characterise the role of the antennae for wind-guided straight-line orientation. In this study, I uncover that the dung beetle antennae carry the main, or likely even sole, mechanosensors for wind orientation. I also find that the loss of one antenna impairs wind sensitivity, but that this only poses a severe issue at low wind speeds; at speeds similar to those experienced on the savannah, beetles with one antenna can still orientate to the wind. Furthermore, I observe no effect on orientation behaviour depending on which antenna (left/right) that is lost. This indicates that *both sensors convey similar directional information in regard to the wind, and that the wind-induced signals in each antenna is summed.* After establishing that the antennae enable wind orientation, I then, in ***Paper II***, turn my attention to the morphology of the putative wind sensor – the antennal Johnston's organ. This intricate stretch receptor detects passive strain, such as that imposed by wind, in the joint between the second and third antennal segments. I find that the individual building-blocks of *K. lamarcki's* Johnston's organ display the same characteristic structures as those identified in other wind orientating species.

On the savannah, the dung beetles have a plethora of directional cues at their disposal. Therefore, the subsequent chapters of the story delve into the integration of directional information given by wind together with a visual cue – the sun. In ***Paper III***, I, together with my colleagues, explore the integration strategy at play which, until our work, was believed to be a "winner-take-all", in which the beetle gauges the usefulness of all available directional cues and solely relies on the cue perceived to be most useful for orientation. However, combining behavioural work with modelling, we demonstrate that the beetles *continuously integrate the directional information given by a simulated wind and sun according to a "weighted vector summation" strategy*. The relative influence of the wind and sun on the combined directional cue is given by their relative weights. Precisely what parameter(s) of a directional cue is considered when weight is afforded is explored in ***Paper IV***, where the dung beetle is exposed to different paradigms of multisensory directional input. These experiments reveal that a combination of directional information from multiple sources does not always generate the most robust behavioural output. We then attempted to replicate my behavioural results using the head direction circuit from ***Paper IV***, by simulating two weighting strategies: *i*) "weight-by-reliability" and *ii*) "weight-by-contrast". As it turns out, the latter weighting strategy best reflected my behavioural data, indicating that *dung beetles do not weigh cues by reliability, but rather by contrast.*

Taken together, the work presented in my thesis demonstrates the dynamic nature of the wind compass, and how it can enable the directed movements of insects, despite the increasing challenges they might face on their diverse journeys across the world.

Populärsammanfattning

När solen strålar ner på den sydafrikanska savannen värms luften upp över sanddynorna, vilket i sin tur bildar tryckgradienter. Dessa gradienter sätter luftmolekylerna i rörelse och driver dem från områden med högtryck till lågtryck – detta är vad du, jag eller en insekt, uppfattar som ***vind***. Medan vinden blåser framåt över sanden kan den bära med sig doften av en dynghög och guida dyngbaggen *Kheper lamarcki* till sin nästa måltid. Hit anländer den ofta i sällskap med hundratals andra hungriga individer. Dessa dyngbaggar formar då en bit dynga till en boll, avvärjer några opportunister som försöker stjäla dyngbollen den nyss skapat, och rullar sedan i väg sin runda måltid – baklänges, med huvudet intill marken och dyngbollen mellan bakbenen. Det mest effektiva sättet att fly konkurrensen vid dynghögen med sin mat i behåll är att hålla en rak kurs bort från dynghögen – vilket är precis det den bollrullande dyngbaggen gör. På så vis maximerar den avståndet mellan sig själv och kaoset vid högen med varje steg den tar. För att bibehålla denna kurs integrerar dyngbaggen olika typer av riktningsinformation i sin *interna kompass*. Denna information kan vara i relation till solen, mönster av himmelsljus eller riktningen vinden blåser från. I den här avhandlingen tar jag hjälp av dyngbaggen *K. lamarcki* för att utforska morfologin och funktionen hos en *vindkompass*.

Berättelsen börjar i ***Artikel I***, där jag karaktäriserar antennernas roll för vindstyrd orientering. I den här studien visar jag att det är dyngbaggens *antenner* som känner av vinden och möjliggör vindorientering. Jag fann även att dyngbaggar med endast en antenn fortfarande kan vindorientera, fast nu med lägre känslighet. Detta indikerar att *båda antenner förmedlar liknande riktningsinformation från vinden, samt att de vindinducerade signalerna i varje antenn summeras*. Efter att ha etablerat att det är antennerna som möjliggör vindorientering tittade jag, i ***Artikel II***, närmre på morfologin hos stretchreceptorn *Johnstons organ*. Denna receptor är förankrad i leden mellan det andra och tredje antennsegmentet och reagerar på passiva rörelser i denna led. Sådana rörelser kan orsakas av vindflöde. I min studie fann jag att byggstenarna som utgör *K. lamarckis* Johnstons organ uppvisar liknande morfologier som de som identifierats hos andra vindorienterande insektsarter. Detta antyder att det är Johnstons organ som möjliggör vindorientering även hos dyngbaggen.

Ute på den sydafrikanska savannen har dyngbaggen en uppsjö av kompass-signaler till sitt förfogande. I Artikel III utforskar jag, tillsammans med mina kollegor, vilken integrationsstrategi dyngbaggen använder för att kombinera riktningsinformation från vinden och solen. Innan vår studie gjordes ansågs strategin vara en så kallad *"winner-take-all"*, vilket innebär att baggen estimerar pålitligheten av alla tillgängliga kompass-signaler och förlitar sig enbart på den som upplevs vara mest pålitlig för orientering i den stunden. Med hjälp av beteendeförsök och modellering visar vi att dyngbaggen *kontinuerligt integrerar den riktningsinformation som ges* *av en simulerad vind och sol* enligt en strategi som kallas *weighted vector summation*, där vindens och solens relativa inverkan på den kombinerade kompass-signalen ges av deras relativa vikter. I ***Artikel IV*** bygger vi vidare på denna modell genom att definiera vilka egenskaper hos kompass-signaler som sätter dessa vikter. Med hjälp av beteendestudier och simuleringar av insektskompassens neurala nätverk finner vi att *dyngbaggar väger riktningsinformation utifrån kompass-signalens relativa kontrast*.

Sammantaget belyser arbetet i min avhandling vindkompassens dynamiska egenskaper. Min beskrivning av de bakomliggande mekanismerna för hur dyngbaggens hjärna kontinuerligt anpassar hur mycket vinden eller solen ska kontrollera i vilken riktning den färdas har tagit oss ännu ett steg på vägen till att förstå hur insekterna färdas runt vår jord. Detta är viktig kunskap att ha med sig då utmaningarna för dessa resor blir allt fler.

1 The starting point of my thesis

Most animals depend on directed movement for survival; from the long-distance migrations of elephants, birds, and monarch butterflies, to the shorter-range orientation of ants, bees, and beetles. To accomplish these navigational feats, animals make use of an intricate *compass system* or *heading indicator* (***Section 2***), that integrates directional information from various sources to guide steering. These sources of information can be internal (*idiothetic*) cues, derived from the animal's proprioceptors as it moves through space. However, internal cues inevitably accumulate errors (i.e. the perceived body position does not reflect reality). In fact, when directional information is limited to internal cues, blindfolded humans tasked with walking a straight trajectory soon deviate from their initial path and begin to circle (Cheung et al., 2007; Souman et al., 2009). Similarly, the compass system of insects, such as dung beetles, naturally accumulates errors in the absence of external visual cues and as a result, the insect also begins to circle when released in full darkness (Khaldy et al., 2019). Thus, to successfully steer along a desired course beyond a few body lengths of distance, the compass system requires further guidance from external (*allothetic*) cues (Cheung et al., 2007; Souman et al., 2009). These external cues are sampled through a range of sensory systems.

In the extensive field of insect navigation, much research has been devoted to unravelling the use of visual compass cues for guidance. Such visual cues include the sun, moon, and stars, as well as spectral- and intensity gradients, and the pattern of polarised light that spans across the sky (Dacke et al., 2021, 2014, 2013, 2012, 2011; el Jundi et al., 2014). However, what happens when these visual cues fail to provide reliable directional information? What other sensory systems work as a back-up to continuously feed the animal's compass systems with the information required to sustain orientation? During conditions where celestial compass cues fail, insects can – given the right conditions – for example steer according to prevailing ***winds*** (Bell and Kramer, 1979; Dacke et al., 2019; Heinzel and Böhm, 1989; Müller and Wehner, 2007; Okubo et al., 2020). This is where my work enters the picture. In this thesis, I use the South African, ball-rolling dung beetle *Kheper lamarcki* as my model organism to provide further insights on wind-guided straight-line orientation. I evaluate the role of the highly mechanosensitive antennae for wind-guided orientation (***Paper I***), and then proceed to characterise the morphology of the wind sensor (***Paper II***). Furthermore, as my model species possesses a dynamic compass system that employs a range of directional cues, I investigate the integration of a visual compass cue with wind (***Papers III*** and ***IV***). Taken together, the work presented in this thesis highlights the versatility of a compass system and its capacity to utilise wind, a continuously moving cue, to guide an animal on its journey.

1.2 The South African, ball-rolling dung beetle

Dung beetles, the world's hardworking groundskeepers, are spread across all continents except for Antarctica. The vast majority of the 7000 species (Coleoptera: Scarabaeinae) feed on the nutrient rich dung left behind by other animals and can be divided into three categories based on their method of consumption: *dwellers* (*endocoprids*), *tunnellers* (*paracoprids*), and *ball-rollers* (*telocoprids*) (Hanski and Cambefort, 1991). Dwellers and tunnellers remain in close proximity to the dung pile and feed directly atop it (dwellers) or from tunnels underneath it (tunnellers). In contrast, the ball-rollers prefer to enjoy their meal at some distance from the dung source. Upon arrival at a fresh dung pile, the beetles are confronted by fierce inter- and intraspecific competition for food (Hanski and Cambefort, 1991). To avoid this, the ball-rolling beetles swiftly break off a piece of dung and sculpt it into a ball, which they then proceed to roll away – backwards and along a straight path (Byrne et al., 2003). To guide their straight-line orientation, the beetle employs a range of compass cues (***Section 2***). By maintaining a relative bearing to these cues, the beetle manages to steer along a straight course. This robust and relatively simple orientation strategy provides the beetle with an escape route wherein each step maximises the distance from its starting point. As a result, this ensures that the beetle does not return to the intense competition at the dung pile. After rolling for approximately 6 minutes (Dacke et al., 2019), the beetle digs down in soft soil to consume its dung ball in peace. A few days later, the beetle has finished its meal and emerges from the ground – hungry and ready to repeat its straight-line escape.

2 Compass cues

As the field of spatial orientation research grows, the explicit definition of a 'compass' has become a matter of debate. Some argue that orientation behaviour can only be considered '*compass orientation*' if the animal can discern absolute compass directions (Grob et al., 2021a; Guilford and Taylor, 2014), while directed behaviour without this type of global frame of reference should rather be referred to as '*non-compass orientation*', or '*straight-line orientation*'. In the latter case, the animal makes use of a '*heading indicator*' to monitor its body orientation relative to the directional cue. If this relationship is maintained as the animal moves, it will steer straight (Grob et al., 2021a; Guilford and Taylor, 2014). This is what we observe in my model animals, the ball-rolling dung beetles (Baird et al., 2010; Byrne et al., 2003). A problem arises if the directional cue is a celestial body, e.g. the sun, that changes its apparent position over the course of a day; a true compass system will compensate for this apparent movement, but a heading-indicator will not, ultimately leading the animal along a curved path. The dung beetles, however, orient for such a short period of time that their straight-line escapes from the dung pile do not suffer from the apparent movement of the sun (Dacke et al., 2019). In scientific reports, the terms 'compass' and 'heading indicator' are often used interchangeably (Freas and Spetch, 2022). In this thesis, I define a compass cue as a cue that an animal can utilise to steer in any direction relative to it (*menotaxis*). The neuronal network that supports the extraction of directional information from the cue is referred to as a compass, e.g. a sun compass or a wind compass, while the term 'heading indicator' refers to the whole network behind steering, without time-compensation or other means to maintain an absolute compass direction. The details of the neuronal circuitry underlying the insect compass are described in ***Section 4***.

2.1 Wind

As sunlight radiates down upon the earth's surface, air is heated at varying intensities; greater at the equator, and dissipating towards the poles (Maceachern and Yildiz, 2018). Where temperatures rise, the density of air molecules decreases. Thus, the uneven heating of Earth by the sun generates relative differences in atmospheric air pressure across space. These pressure gradients drive the movement of air particles from areas of high pressure to areas of low pressure (Kalmikov, 2017;

Mathew, 2007), producing what is experienced by us, or a small insect, as *wind*. Wind can be described by its *velocity*, which is a vector value that defines the speed of airflow (magnitude) and its direction. The *speed* with which the air particles travel is determined by the pressure difference between two points in space – a greater difference produces greater wind speeds. The *direction* of wind is given by the orientation of the pressure gradient.

Wind can either be *laminar* (vectors move in parallel and with constant speed) or *turbulent* (vectors are subject to irregular fluctuations), depending on the dynamic properties of the wind, as well as the environment it is moving through. To predict the behaviour of a fluid – such as air – in a determined environment, the *Reynolds number* is used; this number describes the relative effects of inertial and viscous forces on fluid flow. In experiments conducted by Reynolds (1883), a streak of dye was introduced to a pipe with flowing liquid and the tendency to depart from a uniform stream was used as a visual representation of laminar and turbulent flow. Reynolds (1883) found that the transition from laminar to turbulent could be attained by either **(1)** increasing flow speed, **(2)** increasing pipe diameter, **(3)** decreasing the viscosity of the fluid, or **(4)** increasing the density of the fluid. As a result, he proposed the relationship described in Equation (1), where U is flow velocity, L is characteristic length, and v is the fluid viscosity. A low Reynolds number indicates that viscous forces dominate, resulting in more laminar fluid flow, while a high value suggests greater turbulence (Purcell, 1977; Reynolds, 1883; Smits et al., 2011; Vogel, 1994).

$$Re = \frac{UL}{v} \tag{1}$$

2.2 The boundary layer and fluid flow around cylinders

Initially explored by (Prandtl, 1904), the *boundary layer* and the flow within it can be illustrated by imagining wind travelling over a horizontal, smooth, and solid surface (see Figure 1, from Kreith (1999). Due to friction, the air moving directly against the surface will adhere to it. This results in zero velocity at the surface that is referred to as the *no-slip condition* (Loudon, 2003; Vogel, 1994). At a certain distance from the surface, air will move freely and with a constant velocity, referred to as the *free stream* (Vogel, 1994). The no-slip condition and the free stream form the extremes, and in between is the boundary layer, where air velocity increases with increasing distance from the surface (Mathew, 2007). The velocity vectors of airflow begin as laminar (laminar boundary layer, see Figure 1). However, as the air travels forward (increasing L in Equation (1)), the boundary layer thickness increases, resulting in greater inter-particle interactions which cause disturbed flow. This leads to a transition from a laminar to a turbulent boundary layer.

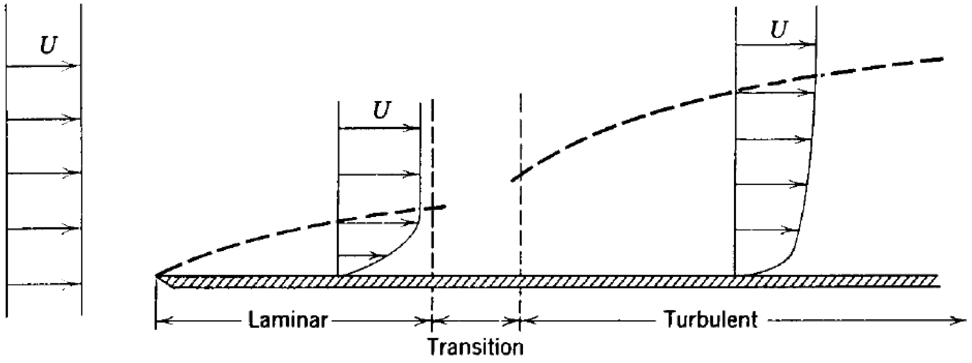

Figure 1: Boundary layer formation as wind travels over a flat surface.
Vectors denote wind direction and speed (U). Depending on the local Reynolds number, at a certain distance from the leading edge, the boundary layer will depart from laminar and enter a transition into turbulent. *From Kreith (1999).*

The transition from a laminar to turbulent boundary layer may occur earlier if obstructions are introduced to the wind flow. The velocity of wind (speed and direction) can undergo rapid fluctuations when subjected to additional frictional forces by e.g. rough ground, vegetation, balls of dung, or other obstacles (Bitsuamlak et al., 2004; Mathew, 2007). These disruptions in airflow reduce the wind speed, resulting in an exponential increase in speed with vertical distance from the textured ground, similar to what was explained above (Geiger et al., 1995). The shapes and sizes of the obstructions also affect the direction that the wind takes by encouraging the formation of vortices (Maceachern and Yildiz, 2018; Vogel, 1994). Vogel (1994) describes the effect of Reynolds number on the formation of vortices as a fluid moves past a cylinder, illustrating that with growing Reynolds number, the propensity for vortices increases; beginning as *attached vortices* that remain near the cylinder. As the Reynolds number increases further, these attached vortices are shed and travel downstream of the cylinder, initially in a structured and repetitive manner (*von Kármán vortex trail*) and eventually in a *turbulent wake* (see Figure 2). Loudon (2003) places this into the context of insect antennae and presents the relationship between Reynolds number, wind speed, cylinder diameter (as a proxy for antenna diameter) and vortex behaviour at a defined wind viscosity (see Figure 3). At Reynolds numbers below 10 (low air speed and large cylinder diameter, or high air speed and small cylinder diameter) the flow is laminar and devoid of vortices. However, as the number increases, the flow goes through a period of attached vortices (10 < Reynolds number < 40), followed by vortex shedding (Reynolds number > 40). The biological implications of attached vortices and the von Kármán vortex shedding could be an added level of noise in the form of unwanted oscillations of the wind sensitive structures.

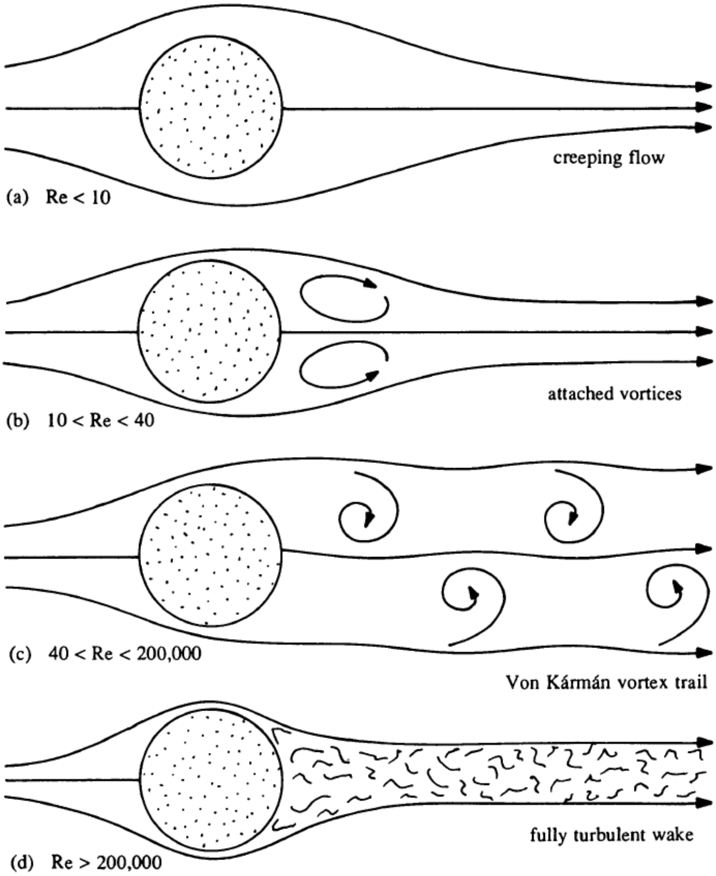

Figure 2: Flow behind a cylinder, illustrating the formation of vortices at different Reynolds numbers.

At low Reynolds numbers, the flow begins as relatively laminar (A). As the number increases, attached vortices form (B) which are shed with increasing Reynolds number into a structured von Kármán trail (C), and later in a turbulent wake (D). *From Vogel (1994).*

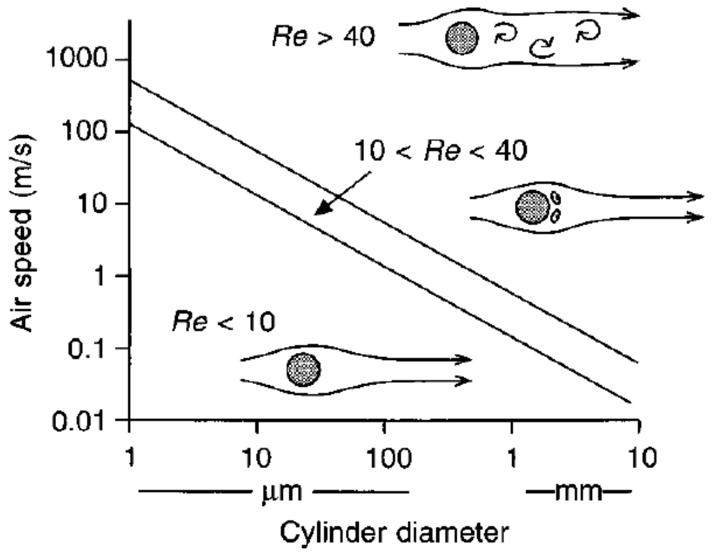

Figure 3: Relationship between air speed (*m/s*), cylinder diameter, Reynolds number and vortex formation behind the cylinder.
At low Reynolds numbers (Re < 10) the flow is relatively laminar. At intermediate Reynolds numbers (10 < Re < 40) there is a formation of attached vortices. At higher Reynolds numbers (Re > 40) these vortices are shed. *From Loudon (2003).*

2.3 Wind is a versatile medium that can facilitate a wide range of behaviours

Despite the complex fluid dynamics of wind, several animals take advantage of prevailing winds to guide their behaviours. In the realm of insects, wind provides a vital medium through which olfactory cues can be transmitted and received for communication, mate-seeking, and foraging (Cardé and Willis, 2008; Collett and Cardé, 2014; Kaissling and Kramer, 1990; Kramer, 1976; van Breugel et al., 2022). Changes in wind flow in the immediate surroundings have been observed to trigger escape responses in crickets (Gnatzy and Heußlein, 1986), caterpillars (Tautz, 1977) and cockroaches (Camhi and Tom, 1978), and several insect species steer by the guidance of the wind flow. These include desert ants (Müller and Wehner, 2007; Wehner and Duelli, 1971), fruit flies (Budick et al., 2008), walking beetles (Heinzel and Böhm, 1984; Linsenmair, 1972) and the South African, ball-rolling dung beetle *Kheper lamarcki* – which utilises the wind *anemomenotactically* (Dacke et al., 2019; ***Papers I, III, and IV***).

2.4 The wind conditions in the beetle's natural habitat support wind-guided orientation

On the South African savannah, the natural habitat of *K. lamarcki*, celestial cues are accessible and useful for guidance throughout most of the day. However, around midday when the sun nears its highest elevations, the directional information given by available celestial cues becomes less useful for orientation (Müller and Wehner, 2007). This event overlaps in part with the peak diel activity hours of this beetle species (Dacke et al., 2019; Tocco et al., 2021). However, despite their now relatively unreliable sun compass, the beetles still maintain their straight trajectories away from the pandemonium at the dung pat. Coincidentally, it is also around this time of day when prevailing wind currents reach their highest speeds (Dacke et al., 2019) (see Figure 4A). Additionally, measurements of angular variation of wind in the dung beetle's habitat show that there is little change in wind direction during a 6-minute-long escape from the dung pat (median angular change = 27°, see Figure 4B, (Dacke et al., 2019)). Taken together, as beetles only steer along their escape bearings for these relatively short periods of time, these conditions favour wind as an alternative cue for guiding straight-line orientation around noon. Indeed, behavioural experiments reveal that as the elevation of the sun increases, directional information from wind is afforded greater relative weight by the beetle's compass (Dacke et al., 2019; ***Paper III***). As directional information can be passed between the wind and sun compasses of the beetles (Dacke et al., 2019), this supports a dynamic orientation system that is able to sustain robust steering away from the dung pile for the entire day.

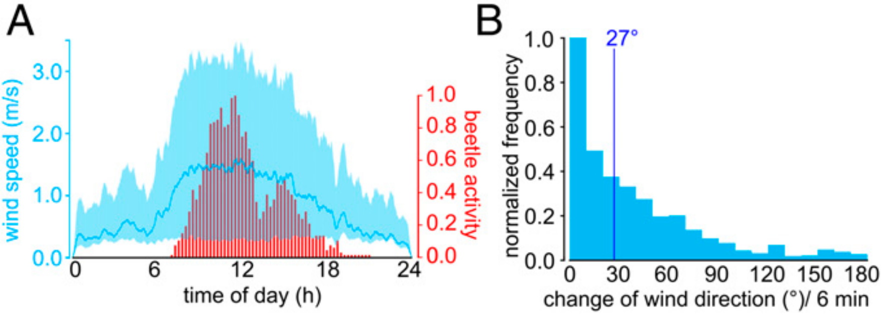

Figure 4: Wind conditions on savannah and beetle activity. (A)
Average wind speeds measured in the natural habitat of *K. lamarcki* (blue axis) and the relative activity of the beetles over a day (red axis). (B) The normalised frequency of wind directional changes over a 6-minute period. Median angular change ± IQR = 27° ± 48.5°. *From Dacke et al. (2019).*

2.5 The reliability of wind as an orientation cue increases with increased speed

To study the characteristics of the dung beetle wind compass in more detail, my colleagues and I designed a behavioural setup where the cues presented to the beetle, including wind, could be carefully controlled (see Box 2, ***Paper III***). To define how the *reliability* of wind as an orientation cue varied across a range of speeds, we used *orientation precision* (the beetle's ability to maintain its set bearing over several consecutive trials) as a proxy. Orientation precision was quantified by calculating the beetles' *mean vector length* (Box 2). We trialled beetles at wind speeds of 0.5, 0.8, 1.0, 1.9, 2.5, 3.0, and 4.0 *m/s* (as measured from the centre of our setup, 1.3 *m* away from the wind generators, using a hot-wire anemometer). To uphold beetle motivation, a simulated sun was always present in zenith – providing illumination, but no directional information.

I found that, as wind speeds increased, so did the orientation precision of the beetles (see Figure 5, ***Paper III***). This suggests that the wind current becomes a more useful orientation cue with increasing speed, as long as it does not blow the beetle off course. Work in walking dung beetles has shown that it is the antennae that detect wind currents (Linsenmair, 1972), and Okubo et al. (2020) have shown that with increased wind speed, the antennae of fruit flies are subject to greater deflections. It may indeed be so that greater deflections of the dung beetle antennae provide a clearer perception of the wind direction.

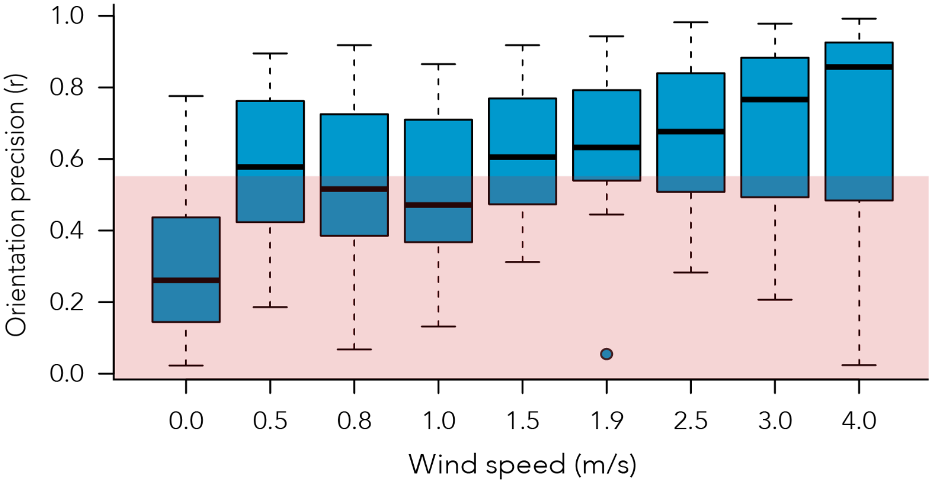

Figure 4: Population orientation precision.
The blue boxes represent the mean vector lengths (used as a proxy for orientation precision, y-axis) of beetles orientating in the presence of a simulated wind at 0.0, 0.5, 0.8, 1.0, 1.5, 1.9, 2.5, 3.0, and 4.0 *m/s* (x-axis). The black bar in each box denotes the median vector length, the whiskers extend to the minimum and maximum values, and blue circles indicate outliers. The pink shaded area illustrates the critical mean vector length, calculated with respect to a significance level of 0.05 (Rayleigh test); if the median mean vector length of the population falls within this area, it signifies that the beetles were unable to maintain a consistent bearing (*n* = 20). Median mean vector lengths [IQR]: 0.0 *m/s* = 0.26 [0.14, 0.44], 0.5 *m/s* = 0.58 [0.42, 0.77], 0.8 *m/s* = 0.52 [0.38, 0.74], 1.0 *m/s* = 0.47 [0.36, 0.72], 1.5 *m/s* = 0.61 [0.45, 0.80], 1.9 *m/s* = 0.63 [0.54, 0.81], 2.5 *m/s* = 0.68 [0.51, 0.84], 3.0 *m/s* = 0.77 [0.47, 0.89], 4.0 *m/s* = 0.86 [0.48, 93]. Adapted from *Paper III*.

These experiments also enabled me to explore a range of wind speeds under which the dung beetle wind compass can support orientation. The beetles performed consistent wind-guided straight-line orientation in the presence of a wind of 0.5 *m/s*. Due to boundary layer effects, the wind speeds close to the ground are relatively low (Vogel, 1994). Therefore, it may be considered unsurprising that these insects, that predominantly inhabit the ground when they orientate, are sensitive to low wind speeds. Among other wind orientating beetles, wind sensitivity thresholds of 0.15 *m/s* have been recorded, and cockroaches can orientate themselves at winds as low as 0.015 *m/s* (Bell and Kramer, 1979; Linsenmair, 1972). From my studies, it can be concluded that the lower threshold for wind-guided orientation in the dung beetle *K. lamarcki* also lies below 0.5 *m/s*.

K. lamarcki continued to perform precise wind-guided straight-line orientation even in the presence of a simulated wind at a speed of 4.0 *m/s*. Similarly, desert ants have been observed to utilise wind currents ranging from 2.0 to 6.0 *m/s* (Müller and Wehner, 2007). Interestingly, when presenting beetles with high wind speeds, I observe a shift in orientation strategy from anemomenotaxis to positive anemotaxis (For details, see ***Section 3***, ***Papers I*** and ***III***). It is important to remember that during its straight-line orientation, the beetle is transporting the large and heavy dung ball backwards with its hind legs, effectively placing it behind the insect. The ball itself might not only obstruct wind input to the wind sensors (***Paper I***), but in the presence of a strong side-wind it may become difficult for the beetle to manoeuvre it in a controlled manner. Thus, orientating upwind when winds are strong may be a strategy utilised by the beetle to ensure that it does not lose touch with its meal.

2.6 Sun

An abundance of animals use the sun to steer a desired trajectory, from early studies in ants (Santschi, 1910) and honeybees (von Frisch and Lindauer, 1956), to more recent work in monarch butterflies (Heinze and Reppert, 2011; Mouritsen and Frost, 2002), fruit flies (Beetz and el Jundi, 2018; Giraldo et al., 2018), sandhoppers (Forward et al., 2009), desert ants (Müller and Wehner, 2007; Wehner and Müller, 2006), and birds (Guilford and Taylor, 2014; Schmidt-Koenig et al., 1991). Sun-guided orientation has also been repeatedly reported for the ball-rolling dung beetles (Byrne et al., 2003; Dacke et al., 2014). Not only is the sun itself a directional cue, but the scattering of sunlight, as it hits the atmosphere, across the sky also gives rise to several celestial compass cues: a light intensity gradient, a chromatic gradient, and a pattern of polarised light.

Over the day, the sun appears to move across the sky. This presents a challenge for animals that travel long distances, as steering exclusively in relationship to the sun over a longer period will unavoidably result in a curved path. Birds, monarch butterflies and hoverflies are three, among many, well known long-distance migrators whose journeys may span over several weeks. To successfully find their way, these animals employ time compensation mechanisms to correct for its apparent motion of the sun (Guilford and Taylor, 2014; Massy et al., 2021; Mouritsen and Frost, 2002; Reppert et al., 2010). Clock shift experiments reveal that these compensatory mechanisms depend on the animal's circadian clock, which in butterflies is believed to rely on clock proteins in their antennae (Guerra et al., 2012; Merlin et al., 2009). During the short times (on average 6 minutes) that the dung beetle rolls its ball (Dacke et al., 2019), the sun's position does not change markedly, making it an excellent cue to guide their short distance straight-line orientation.

Box 1: Behavioural setup – simulated sun and wind

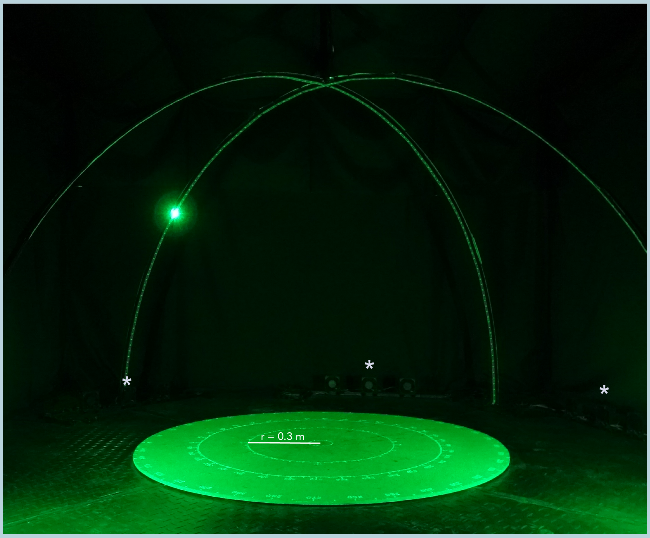

Figure B1: Simulating sun and wind. Behavioural setup consisting of two metal arches lined with green LEDs (520 *nm*, each simulating a sun), wind generators (asterisks), and a circular, wooden arena with its perimeter labelled from 0-355°. *From Paper III*.

Two metal arches were crossed to form a hemisphere. Both arches were lined with 141 individual LEDs each (520 *nm*, DotStar; Adafruit Industries, New York, USA). Each LED served as an isolated ersatz sun with an intensity of 2 × 1011 *photons/*cm^2*/sec* as measured from the centre of the setup and at a height of about 7 *cm* (corresponding to the height of a beetle when atop its dung ball, QE65000; Ocean Optics). Four wind generators, consisting of three individual fans (PFR0912XHEE, 4.50*A*; Delta Electronics Inc., Taipei City, Taiwan) distributed evenly over 1.0 *m*, were positioned 1.3 *m* from the centre of the setup and distributed with an angular separation of 60°. A hot wire anemometer (HHF-SD1; Omega), kept at a height of 7 *cm*, was used to measure the wind speeds at the centre of the setup. Placed in the centre of the setup was a circular, sand-painted arena (r = 0.3 *m*), with its perimeter labelled from 0-355°, with 0° aligned with magnetic north. The elevation of the ersatz sun and the wind speed were controlled using a custom-built software in conjunction with a Raspberry Pi 4 Model B. All experiments were filmed using a Sony camera (FDR-AX53 Handycam) mounted on a tripod above the arena. To remove any unwanted orientation cues, the setup was placed inside a 3x3 *m* tent constructed from blackout cloth.

The usefulness of the sun as an orientation cue is highly reliant upon its elevation and the environmental conditions. In ***Paper III***, my colleagues and I evaluated the orientation precision of beetles in the presence of a simulated sun at the elevations 5°, 20°, 45°, 60°, 75°, 80°, 82°, 84°, 86°, 88° and 90°. We found that orientation precision remained high until a 75° solar elevation, after which it declined markedly (see Figure 6). This demonstrates that with increasing elevation, the directional information given by a simulated sun becomes increasingly ambiguous, and in turn its usefulness as a directional cue decreases. In instances when the sun does not provide reliable directional information, the beetle must turn to alternative guidance cues – such as wind.

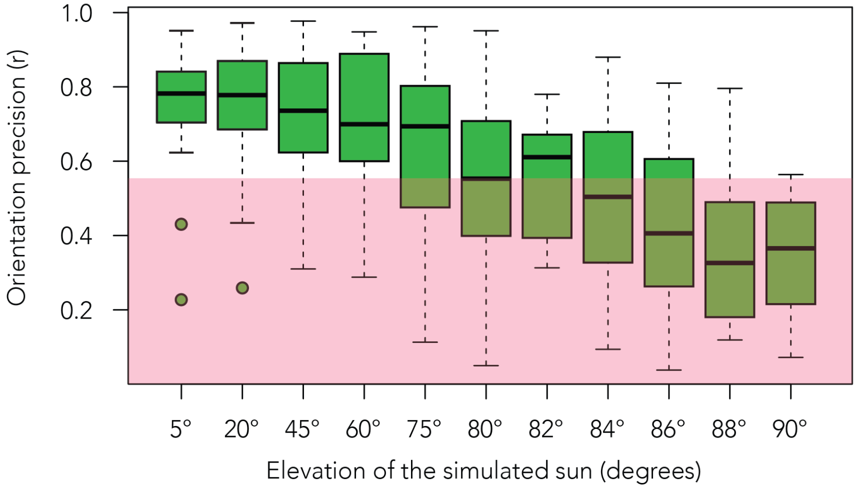

Figure 6: Population orientation precision. The green boxes represent the mean vector lengths (used as a proxy for orientation precision, y-axis) of beetles orientating in the presence of a simulated sun at 5°, 20°, 45°, 60°, 75°, 80°, 82°, 84°, 86°, 88° and 90° (x-axis). The black bar in each box denotes the median vector length, the whiskers extend to the minimum and maximum values, and the green circles indicate outliers. The pink shaded area illustrates the critical mean vector length, calculated with respect to a significance level of 0.05 (Rayleigh test); if the median mean vector length of the population falls within this area, it signifies that the beetles were unable to maintain a consistent bearing (*n* = 20). Median mean vector lengths [IQR]: 5° = 0.78 [0.71, 0.84], 20° = 0.78 [0.69, 0.86], 45° = 0.74 [0.64, 0.84], 60° = 0.70 [0.61, 0.88], 75° = 0.69 [0.48, 0.80], 80° = 0.55 [0.40, 0.70], 82° = 0.61 [0.40, 0.66], 84° = 0.50 [0.34, 0.67], 86° = 0.41 [0.27, 0.60], 88° = 0.33 [0.19, 0.48], and 90° = 0.37 [0.22, 0.49]. *Adapted from **Paper III***.

3 The structure and function of the antennae

One of the most noticeable paired sensors across insect species is their *antennae*. Approximately 13 distinct morphologies of insect antennae have been identified (Borror et al., 2005), but their basic divisions are the same; closest to the head of the insect is the *scape*, followed by the *pedicel* and the *flagellum* (Borror et al., 2005; Donley et al., 2022; Schneider, 1964). As can be expected, the antennae of the South African, ball-rolling dung beetle *Kheper lamarcki* also follow to this typical division into scape, pedicel and flagellum (see Figure 7, ***Paper II***). The beetle's flagellum, the third antennal segment, is comprised of four *flagellomeres* and a distal *club*. The latter is further divided into plate-like lobes, that the beetle can extend laterally in a fan-like fashion, effectively increasing the surface area of this tiny structure. These *lamellate* antennae (Borror et al., 2005) are characteristic for the family Scarabaeidae.

Among insects, antennae are probably best known as olfactory sense organs (Dethier et al., 1963; Frisch, 1922; Hansson and Stensmyr, 2011; Schlief and Wilson, 2007; Schneider, 1964; Suzuki, 1975). Given their preferred meal, it does not come as a surprise that the antennae of the dung-foraging *K. lamarcki* are covered in hairs, presumably serving an olfactory purpose (see Figure 7, ***Paper II***). However, this is yet to be validated. Furthermore, insect antennae have also been suggested to be involved in graviception (Kamikouchi et al., 2009; Matsuo and Kamikouchi, 2013), hygroception (Yokohari et al., 1982), thermoreception (Tichy, 1979) and the monitoring of circadian rhythms (Brady et al., 2021; Merlin et al., 2009). In addition to this, several studies report their role as *airflow sensors* (Gewecke, 1974, 1970; Heinzel and Gewecke, 1987; Yack, 2004); a function involved in head stabilisation and flight control (Chatterjee et al., 2022; Dahake et al., 2018; Sane et al., 2007). In this section of my thesis, I will focus on their role for wind-guided orientation (Birukow, 1958; Böhm, 1995; Linsenmair, 1972).

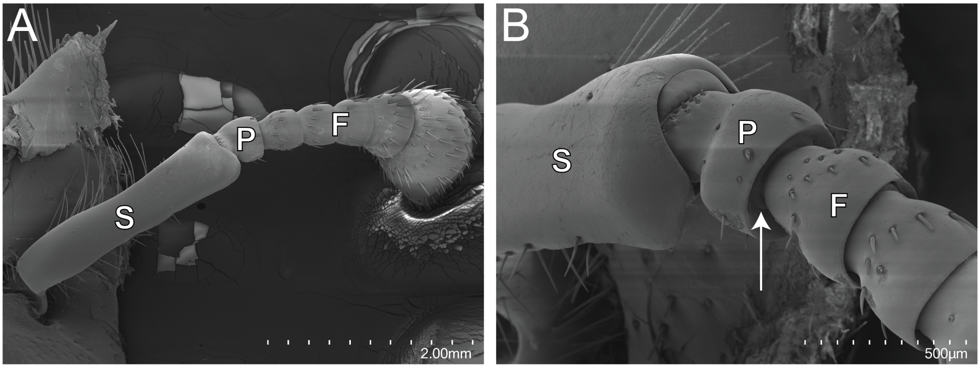

Figure 7: Scanning electron micrographs of the *K. lamarcki* antenna. (A) The three segments of the lamellate antennae: scape (S), pedicel (P) and the flagellum (F). (B) The scape-pedicellar and pedicellar-flagellar joints. The arrow indicates the input region of the Johnston's organ. *Unpublished data (Paper II).*

3.1 The antennae carry the primary sensors for wind-guided orientation in the dung beetle *K. lamarcki*

Given that all wind orientating insects studied to date, including running beetles and carrion beetles (Birukow, 1958; Heinzel and Böhm, 1989; Linsenmair, 1972), cockroaches (Bell and Kramer, 1979), desert ants (Müller and Wehner, 2007), and fruit flies (Okubo et al., 2020; Yorozu et al., 2009) decode the directional component of wind with their antennae, this morphological structure has naturally been proposed to serve an important role for wind-guided orientation also in *K. lamarcki* (Dacke et al., 2019 and ***Paper III***). To better define the role of the antennae for wind-guided straight-line orientation in dung beetles, I set out to characterise the orientation precision of beetles with *two*, *one* and *no* antennae in response to simulated wind currents at speeds of 0.5 and 2.5 *m/s* (***Paper I***). Since the beetle's motivation to orientate declines in complete darkness, the experiments were conducted with a simulated sun at a 90° elevation – providing light, but no directional information (***Paper III***). Orientation precision was determined by calculating each beetle's mean vector length (*r*-value) from ten exit angles. I also evaluated the *straightness* of the beetles' trajectories (quantified from the ratio between the radius of the experimental arena and the beetle's path). The antennal ablations were performed at the base of the scape one day prior to experimentation. All experiments were conducted in our tightly controlled indoor setup (Box 1, page 27).

Provided with simulated wind at a speed of 2.5 *m/s*, the beetles were able to repeatedly exit the arena along straight, well directed paths (see Figure 8, dark blue boxes, see Figure 9, ***Paper I***). After a full ablation of their antennae, the orientation precision of the beetles fell to values comparable to those reported for beetles orientating without any wind at all (***Paper III***). When presented with the directional information of a simulated sun at a 45° elevation, the individuals with *no* antennae regained their ability to steer along their chosen bearings (see Figure 8, green box), clearly showing that with the relevant compass cues available these beetles are still able to perform straight-line orientation. The findings from ***Paper I*** thus confirm that the antennae serve a critical role for the well documented wind-guided orientation of *K. lamarcki*. For details regarding the exact mechanosensory organ that underlies wind sensing, see ***Section 3.5*** and ***Paper II***.

3.2 Beetles with one antenna display a reduction in wind sensitivity

After concluding that beetles without their antennae do not orientate using the wind, I turned my attention to the mechanism behind the extraction of directional information from the wind. I found that in the presence of a wind current of 2.5 *m/s*, the removal of *one* antenna led to a subtle, non-significant decrease in both orientation precision and path straightness (see Figures 8 and 9). This demonstrates that albeit the beetles benefit from having *two* antennae, those with *one* antenna are still able to steer consistently in the presence of a wind current of 2.5 *m/s*. Similarly, dung beetles of the genus *Geotrupes*, and *Blatella* cockroaches are also able to perform consistent wind-guided orientation with *one* antenna (Bell and Kramer, 1979; Birukow, 1958; Linsenmair, 1972). Furthermore, I found no influence on orientation behaviour based on whether the left or right antenna had been removed (see Figure 10, right circular plot, orange and purple data points), which suggests that each antenna relays similar wind-induced signals. The details underlying an insect's ability to extract directional information from wind with only *one* sensor is yet to be unravelled in full. However, preliminary electrophysiological recordings from hawkmoth antennae indicate that the individual sensory units of the chordotonal Johnston's organ, a known wind sensor (see ***Section 3.5.*** and ***Paper II***), exhibit directional tuning (Dieudonne et al., 2014). This could enable an animal to discern wind direction with only *one* antenna. How the sensory units of *K. lamarcki*'s Johnston's organ responds to wind from different directions is a topic for future investigation.

Although dung beetles with *one* antenna continue to consistently steer along their chosen bearings in the presence of a wind current of 2.5 *m/s*, the loss of one sensor is not completely unproblematic. When guided by winds of 0.5 *m/s*, the beetles with *one* antenna could no longer reliably steer in a given direction (see Figure 8, pale blue boxes). To summarise, my results clearly show that beetles with *one* antenna can extract directional information from wind at 2.5 *m/s*, but not at 0.5 *m/s*, suggesting that the wind-induced signals from the two sensors are summed. Interestingly, extensive work on the neural circuitry underlying wind-encoding in *Drosophila melanogaster* reveal that integration of wind-induced information from *both* antennae is necessary to accurately gauge wind direction (Okubo et al., 2020) (see ***Section 4*** of this thesis for details). To fully understand the relationship between the dung beetle's antennae, as well as how its wind-encoding pathway might differ from the fruit fly, electrophysiological experiments are needed.

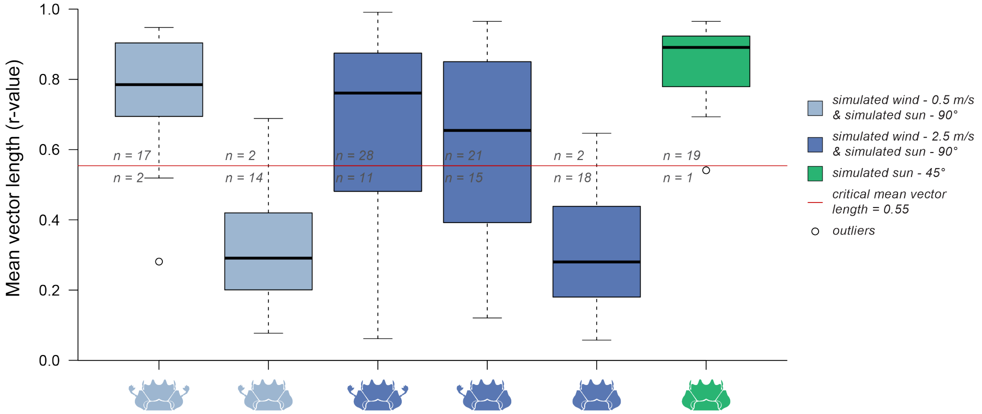

Figure 8: Population orientation precision.
Boxplots represent the mean vector lengths (used as a proxy for orientation precision) of beetles with *two*, *one* and *no* antennae (see schematics on x-axis) orientating in the presence of simulated winds at 0.5 *m/s* (pale blue boxes), 2.5 *m/s* (dark blue boxes), or 0 *m/s* with a simulated sun at a 45° solar elevation (green box). The black bar in each box represents the median *r*-value, the whiskers extend to the minimum and maximum values, and the open circles indicate outliers. The red line marks the critical mean vector length, calculated with respect to a significance level of 0.05 (Rayleigh test); the fraction of beetles with a distribution of exit angles that exceeded this critical mean vector are indicated above the red line, while the fraction of beetles with a distribution of exit angles that fell *below* are indicated below the red line for each experimental condition. *Unpublished data (Paper I).*

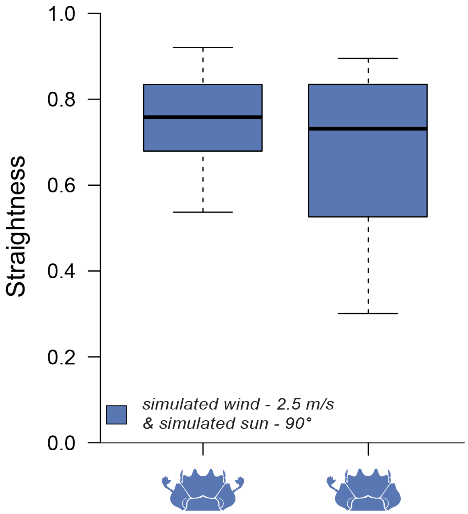

Figure 9: Straightness of trajectories.
Boxplots represent path straightness (calculated as the ratio between the direct distance between the centre and exit point on the arena (r = 0.3 *m*) and the length of the beetle's path) of beetles with *two* antennae and *one* antenna orientating in winds of 2.5 *m/s* (dark blue boxes, $n = 13_{\text{two antennae}}$, $n = 10_{\text{one antenna}}$). The black bar in each box represents the median straightness of each population and the whiskers extend to the minimum and maximum values. *Unpublished data (**Paper I**).*

3.3 Beetles adjust their orientation strategy based on wind speed and antennal condition

As briefly touch upon in ***Paper III***, dung beetles adjust their wind orientation strategy depending on wind speed; at low speeds, beetles display a tendency to perform *negative anemotaxis*, at medium wind speeds they perform *anemomenotaxis*, and at high speeds they display *positive anemotaxis*. I expand on this finding in ***Paper I***, where beetles with *two* antennae again are observed to consistently orientate downwind in the presence of 0.5 *m/s* wind, i.e. performing negative anemotaxis (see Figure 10, left circular graph). It is important to remember that when transporting their dung balls, the beetles move backwards with the ball secured between the second and third pair of legs. Therefore, by moving downwind, the antennae remain exposed towards the source of the weak wind, which should – at least in theory – effectively maximise the wind input to the sensors. Similarly, when presented with low wind speeds, slugs that use wind currents for olfactory orientation extend their tentacles towards the current (Kalmus, 1942), and cockroaches tend to run upwind at low wind speeds (0.03-0.06 *m/s*) (Bell and Kramer, 1979). It should be noted that at a wind speed of 0.5 *m/s*, bearing preference in relationship to the wind was not analysed for beetles with *one* antenna, as these individuals were unable to orientate themselves in the presence of a wind at that speed. I found no directional preference in relationship to a simulated wind at 2.5 *m/s* among beetles with *two* antennae, i.e. at this speed of the wind the beetles were performing *menotaxis* (see Figure 10, middle circular graph). Dung beetles are known to orient menotactically in response to wind (***Paper III***), and this is a strategy that certainly works in their favour. At a dung pile, the competition for food is high and beetles are not above resorting to stealing other individuals' balls of dung. However, by orientating menotactically (i.e. in any direction in relationship to the compass cue) as they roll their dung balls away from the pile, the beetles reduce the likelihood of encountering another ball-rolling beetle that could potentially steal its meal. In addition, as the beetles spread in all directions, they also effectively avoid direct competitions for suitable places to bury down with their balls of dung.

Following the experiments detailed above, I proceeded to ablate *one* antenna from the beetles and, again, evaluated directional preference in relationship to a 2.5 *m/s* simulated wind. Interestingly, I now found a tendency of beetles with *one* antenna to travel upwind in the presence of a 2.5 *m/s* wind, i.e. they now orientated in accordance with *positive anemotaxis* (see Figure 10, right circular plot). As reported in ***Paper III***, beetles with *two* antennae also display positive anemotaxis when orientating to a simulated wind of 3.0 *m/s*. This means that beetles with *one* antenna orientating to a wind of 2.5 *m/s* behave as beetles with *two* antennae orientating in the presence of a stronger 3.0 *m/s* wind. I did not expect these results, and had rather anticipated beetles with *one* antenna orientating to a 2.5 *m/s* wind to display a behaviour that mirrors the negative anemotaxis of beetles with *two* antennae at 0.5 *m/s* wind. This raises the question of what exactly triggers the shift in wind orientation strategy (*negative anemotaxis*, *menotaxis*, or *positive anemotaxis*). One, highly speculative, hypothesis is that rather than wind speed, beetles select their orientation strategy on the basis of perceived noise. As wind speed increases so does the propensity for more turbulent airflow (Purcell, 1977; Reynolds, 1883; Vogel, 1994). Therefore, it could be hypothesised that, relative to 2.5 *m/s*, a wind current at a 3.0 *m/s* speed is turbulent enough to contain a level of noise that triggers positive anemotaxis. Assuming that the loss of *one* antenna decreases signal-to-noise ratio, it may be that the level noise experienced by beetles with *one* antenna at 2.5 *m/s* mirrors the noise experienced by beetles with *two* antennae at 3.0 *m/s*, consequently eliciting the tendency for upwind orientation.

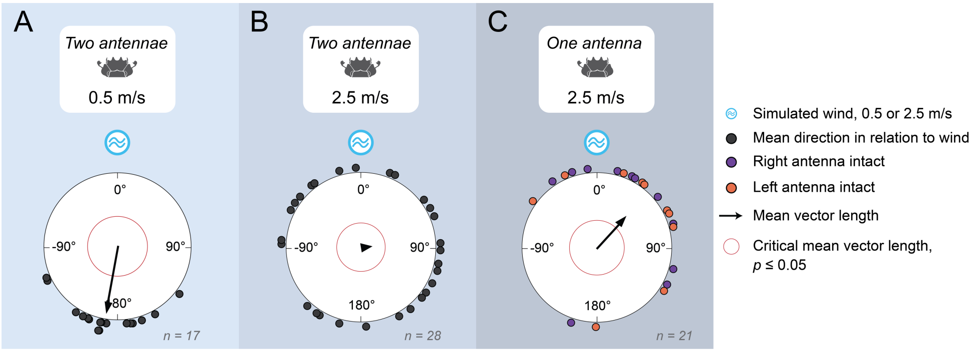

Figure 10: Directional preferences in relationship to wind direction was analysed for beetles whose ten exit angles differed significantly from uniformity ($p \leq 0.05$, Rayleigh test). All angles are standardised to a wind direction of 0°. Directional preferences of beetles with **(A)** *two* antennae orientating at winds of 0.5 *m/s*, **(B)** *two* antennae orientating at winds of 2.5 *m/s*, or **(C)** *one* antenna orientating at winds of 2.5 *m/s*. Each data point represents the mean direction of a single beetle; colour denotes antennal condition – black: beetles with *two* antennae, purple: beetles with right antenna intact, orange: beetles with left antenna intact. The arrow extending from the centre of each circular plot illustrates the mean direction of the population. The length of the arrow denotes the mean vector length. The red circle in each plot represents the critical mean vector length ($p \leq 0.05$, Rayleigh test). *Unpublished data (**Paper I**).*

3.4 Antennal ablations do not impair the beetles’ ability to perform accurate straight-line orientation when guided by a visual directional cue

Not only do insect antennae support wind detection, but they are also known gravity sensors (Kamikouchi et al., 2009; Matsuo and Kamikouchi, 2013). Therefore, in ***Paper I***, in parallel to the wind experiments detailed above, I also investigated the effect of the antennal ablations (*two*, *one*, *no* antennae) on the beetles’ ability for directed movements. In the sole presence of a simulated sun at a 45° solar elevation, irrespective of antennal condition, the beetles continued to orientate along remarkably straight paths. These results clearly demonstrate that, when guided by a simulated sun, antennal ablations do not impose any marked issues to the beetles’ ability to steer straight. While insect antenna can act as graviceptors, they are not the sole sensors that have this function (Frye, 2009). In fact, there are multiple other mechanosensors that serve the same purpose, including cercal hairs, (Horn and Bischof, 1983; Horn and Föller, 2001), halteres (Daltorio and Fox, 2018), campaniform sensilla and prosternal organs (Frye, 2009). Furthermore, stretchreceptive organs similar to the Johnston’s organ are not exclusive to the antennae, and can exist in other joints across an insect’s body (Field and Matheson, 1998; Moulins, 1976). While we do not yet know the identity of *K. lamarcki*’s additional gravity sensors, my results strongly indicate that these animals do at least not rely heavily on their antennae for locomotory control.

3.5 The dung beetle antennae contain a Johnston's organ that shares its morphology with other wind-orienting insects

My results in ***Paper I*** clearly show that the dung beetle *K. lamarcki* relies on its antennae to extract directional information from a simulated wind for wind-guided straight-line orientation. This inspired me to focus on the morphology of the putative wind sensor (***Paper II***). Originally described as the hearing apparatus of mosquitoes by (Johnston, 1855), the chordotonal *Johnston's organ* has since been the object of much fascination. The Johnston's organ is a highly sensitive mechanoreceptor that is composed of multiple, individual sensory units – *scolopidia* – arranged radially within the second antennal segment (the *pedicel*, see Figure 7). The number of scolopidia within the Johnston's organ is variable across insect species, ranging from 19 in miniature wasps (Diakova et al., 2022), 40 in desert ants (Grob et al., 2021b), to roughly 150-200 in fruit flies (Kamikouchi et al., 2006; Todi et al., 2004), and an impressive 7000 in mosquitoes (Boo and Richards, 1975a). The input region of each scolopidium is anchored in a ring of suspension fibres within the pedicellar-flagellar joint (Field and Matheson, 1998; Moulins, 1976). Together, the scolopidia detect passive displacements of the flagellum relative to the pedicel (Field and Matheson, 1998; Kavlie and Albert, 2013; Linsenmair, 1972; Moulins, 1976). Such deflections may be caused by wind. In other species of dung beetles, as well as in cockroaches, immobilisation of the pedicellar-flagellar joint eliminates the insect's ability to orientate using wind (Bell and Kramer, 1979; Birukow, 1958; Linsenmair, 1972). Based on these findings, the Johnston's organ is a convincing candidate as the main antennal wind sensor.

The ultrastructure of the antennal Johnston's organ has been unravelled in mosquitoes (Boo and Richards, 1975; Boo and Richards, 1975), sawflies (Hallberg, 1981), thrips (Bode, 1986), stinkbugs (Jeram and Pabst, 1996), leafhoppers and planthoppers (Stacconi and Romani, 2013), ground beetles (Di Giulio et al., 2012; Todi et al., 2004), fruit flies (Todi et al., 2004), and miniature wasps (Diakova et al., 2022). From histological sections of the pedicel of *K. lamarcki*, I was also able to identify the Johnston's organ in this species (***Paper II***, see Figure 12). As in all insects studied to date, the scolopidia of the dung beetle's Johnston's organ are anchored in a ring of suspension fibres that connect the proximal end of the flagellum to the distal portion of the pedicel. As a result of this arrangement, deflection of the flagellum will impose strain on the input region of the scolopidia, which in turn initiates downstream signalling. Furthermore, I found that the dung beetle scolopidia are grouped into roughly 110 clusters (also termed *scoloparia*, see Figures 12 and 13A, B and C), with 1-5 scolopidia per cluster. Similar to this, mosquitoes also exhibit a variable number of scolopidia per cluster, but the functional significance of these variations are not fully understood (Boo & Richards, 1975). Sawflies display sex-dependent variability in the number of scolopidia per cluster (Hallberg, 1981), but whether this sexual dimorphism also exists in dung beetles, or indeed, if it carries any ecological significance, remains a topic for future investigation.

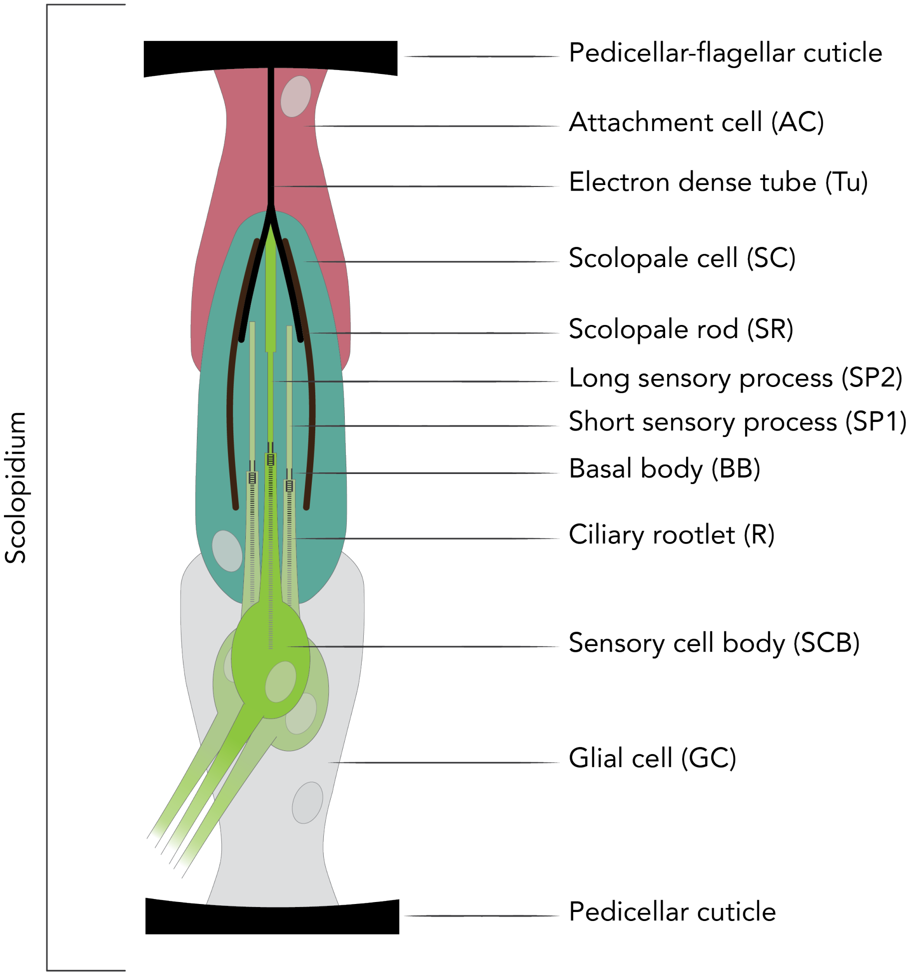

Figure 11: Schematic of a dung beetle scolopidium. Each scolopidium is anchored to the pedicellar-flagellar joint through an electron-dense tube (Tu) and its surrounding attachment cell (AC). Distally, the Tu envelops the long sensory process (SP2) and the shorter sensory processes (SP1), and is embedded within the scolopale rods (SR). The SRs are produced by the scolopale cell (SC) and create a sleeve that surrounds the SP2 and SP1s. At the junction between the cilium and dendrite of each bipolar sensory cell, we find the basal body (BB), as well as the ciliary root (R), of which the latter extends towards the sensory cell-body. A glial cell (GC) secures the sensory cells to the pedicellar cuticle.

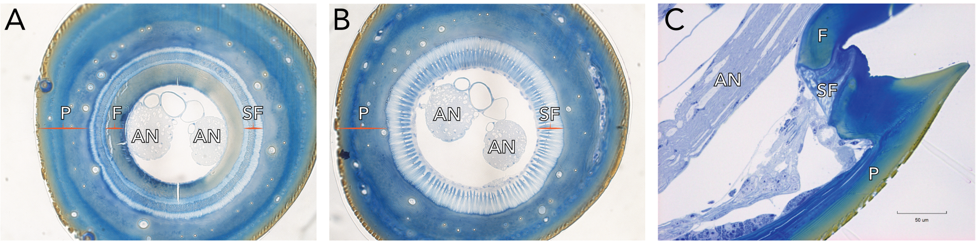

Figure 12: Light micrographs of sections through the pedicel.
(A) A cross-section of the ring of suspension fibres (SF) that link the pedicel (P) to the flagellum (F), the two branches of the antennal nerve (AN), and trachea. (B) A more proximal cross-section, with clusters of scolopidia embedded within the suspension fibres. (C) Longitudinal section of a group of scolopidia that anchor into the suspension fibres. *Unpublished data (Paper II).*

Despite the diversity in Johnston's organ size among insects, the ultrastructure of each scolopidium remains largely constrained across the species studied to date (see Figure 11 for a schematic scolopidium of *K. lamarcki*). The characteristic building-blocks of a single scolopidium are as follows: **(1)** an *attachment cell*, which fixes the scolopidium to the pedicellar-flagellar joint, **(2)** a *scolopale cell* that secretes a barrel-shaped sleeve of electron-dense *scolopale rods* that transmit the mechanical stimuli to the **(3)** *bipolar sensory cells*, whose ciliated dendrites are embedded in the barrel-shaped sleeve of scolopale rods. The sensory cell-bodies are secured to the pedicellar wall by **(4)** *glial cells*. These four cell types are consistently found within insect scolopidia, including the dung beetle, but do not always manifest in identical arrangements (Field and Matheson, 1998; Moulins, 1976).

I further found that in the Johnston's organ of the dung beetle, each scolopidium is fixed to the pedicellar-flagellar joint via an electron-dense tube and its enveloping attachment cell (see Figures 13D and 14A). Distally, this tube stretches into a thread-like structure and inserts into the suspension fibres. This has also been observed in fruit flies (Uga and Kuwabara, 1965), leafhoppers (Howse and Claridge, 1970), tobacco hawkmoths (Vande Berg, 1971), stinkbugs (Jeram and Pabst, 1996; Stacconi and Romani, 2013), planthoppers (Stacconi and Romani, 2013), and microwasps (Diakova et al., 2022) and is characteristic for *amphinematic* scolopidia. *Mononematic* scolopidia, in which each sensory unit is anchored to the pedicellar-flagellar joint via an electron-dense cap, have been identified in mayflies (Schmidt, 1974), and mosquitoes display both amphinematic and mononematic scolopidia (Boo & Richards, 1975; Boo & Richards, 1975). The functional implications of this electron-dense cap are not fully understood, (Field & Matheson, 1998; Moulins, 1976), but does not appear critical for the sensation of wind induced strain.

In addition to dividing scolopidia based on the morphology of their attachment into the pedicellar-flagellar joint, further divisions are made based on the number of bipolar sensory cells and the structure of their ciliated dendrites (Moulins, 1976).

The scolopidia of the dung beetle's Johnston's organ contain *three bipolar sensory cells*, which is a common organisation among the insects studied to date (see Table 9.1. in Moulins (1976)). Furthermore, I observe two types of sensory processes among *K. lamarcki*'s three bipolar sensory cells: two shorter processes (SP1) and one long process (SP2). These sensory processes in turn display structural differences in their dendritic cilia: two of *Type 1* (extends into the scolopale space, uniform diameter, and an orderly array of microtubules), and one of *Type 2* (extends into the electron-dense tube, variable diameter, unorganised array of microtubules). This arrangement is shared with a range of insects (Boo & Richards, 1975; Boo & Richards, 1975; Di Giulio et al., 2012; Diakova et al., 2022; Moulins, 1976; Stacconi & Romani, 2013; Vande Berg, 1971).

The functional significance of the two cilia types remains unexplored. It has been suggested that the structural differences affect the type of stimulus that is detected (Field and Matheson, 1998; Moulins, 1976; Stacconi and Romani, 2013). In fact, fruit flies (Kamikouchi et al., 2009; Yorozu et al., 2009) and hawkmoths (Sane et al., 2007) display distinct groups of JO sensory cells that are tuned to specific frequencies; some are sensitive to high-frequency antennal vibrations (e.g. caused by acoustic signals), while others rather respond to low-frequency stimuli, e.g. wind-induced deflections. Though dung beetles are not known to orientate to acoustic signals, they do detect the vibrations caused by their developing larvae (Hanski and Cambefort, 1991). And as shown in ***Papers I***, ***III*** and ***IV***, *K. lamarcki* is capable of detecting and orientating to a wind stimulus. Further investigation is necessary to better understand if the differences in cilia structure play a role in which stimulus is detected by each sensory cell.

Taken together, my work in ***Paper II***, reveals that the morphology of the dung beetle Johnston's organ shares many of its characteristics with those identified for other insects. Its scolopidia are grouped into approximately 110 clusters, with 1 to 5 scolopidia per cluster. This first detailed morphological description of the dung beetle Johnston's organ provides a solid foundation for further characterisation of its role in the decoding of wind direction.

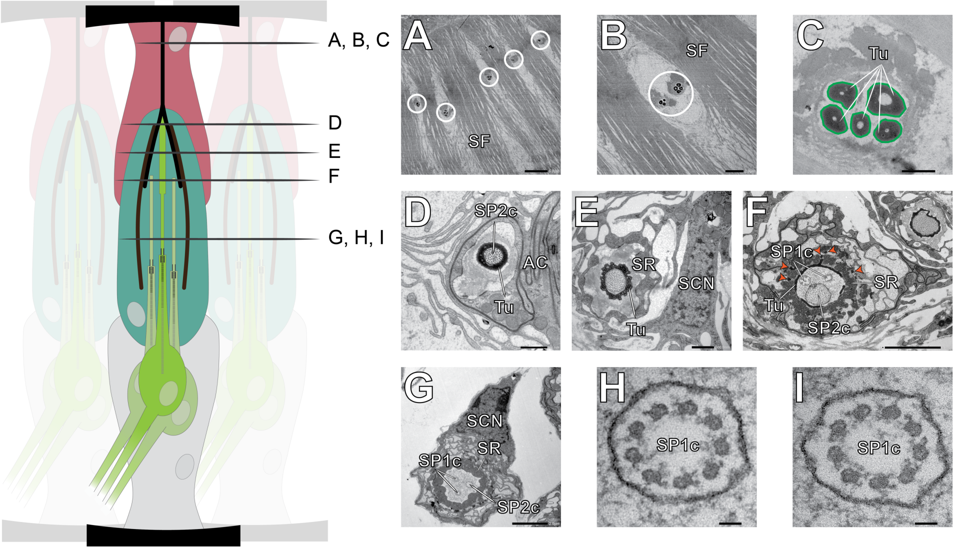

Figure 13: Serial cross-sections of scolopidia in the pedicel of the dung beetle *K. lamarcki*.
Left panel: schematic scolopidia with positions of cross-sections denoted by letters corresponding to the right panel. (A) Five clusters (white circles), each with varying scolopidia number, supported by suspension fibres (SF). Scale bar 5 *µm*. (B) Two clusters (white circle), with three and four scolopidia respectively. Scale bar 2 *µm*. (C) A single cluster with five scolopidia (green outlines), each electron-dense tube (Tu) is associated with one scolopidium. Scale bar 500 *nm*. (D) The attachment cell (AC) enveloping the Tu, in which the cilia of the long sensory process (SP2c) is sheathed. Scale bar 500 *nm*. (E) At a more proximal level, the Tu appears more ruffled and the ring of scolopale rods (SR) surrounding the sensory processes comes into view. The SRs are secreted by the scolopale cell (SCN) which displays the vacuolated structure characteristic of secretory cells. Scale bar 500 *nm*. (F) Proximally, the cilia of two additional sensory processes (SP1c) – which are shorter and have a smaller diameter compared to the SP2c – emerge in the scolopale space, the Tu begins to disappear, and electron-dense material that is visually similar to the Tu is interspersed in the vacuolated scolopale cell (orange arrowheads). Scale bar 2 *µm*. (G) Proximal to the sensory cell bodies, the axonemes of the two SP1 cilia display a 9+0 microtubuli doublet arrangement, the diameter of the SP2c decreases and its microtubuli remains unorganised, the Tu no longer envelops the cilia. Scale bar 2 *µm*. (H, I) Closer images of the two SP1 cilia in figure (G), clearly showing the 9+0 microtubuli doublets characteristic of these cilia. Scale bar 50nm. *Unpublished data (Paper II)*.

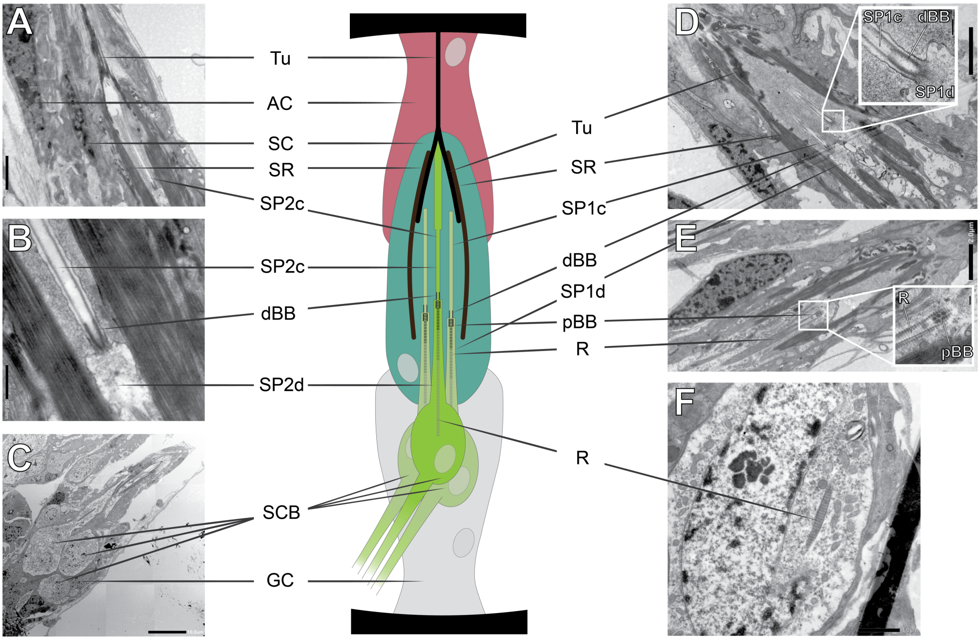

Figure 14: Longitudinal sections of scolopidia in the pedicel of the dung beetle *K. lamarcki*. Central panel: a schematic scolopidium inferred from the electron micrographs acquired for this study. (A) The electron-dense tube (Tu), the attachment cell nucleus (AC), the scolopale cell nucleus (SC), scolopale rods (SR), and the Type 2 cilium of the long sensory process (SP2c). Scale bar 2 *µm*. (B) The Type 2 cilium of the long sensory process (SP2c), clearly showing the distal basal body (dBB) and the dendrite (SP2d). Scale bar 500 *nm*. (C) Overview micrograph of scolopidia demonstrating the cell bodies of the bipolar sensory cells (SCB) and the supporting glial cell (GC). Scale bar 10 *µm*. (D) Electron-dense tube (Tu), scolopale rods (SR), Type 1 cilia of the short sensory processes (SP1c), the dendrite of the short sensory processes (SP1d), distal basal body (dBB). Scale bar 2 *µm*. The inset shows the cilium-dendrite junction. Scale bar 200 *nm*. (E) The proximal basal body (pBB), and the ciliary root (R). Scale bar 2 *µm*, the inset highlights the pBB and the R. Scale bar 200 *nm*. (F) The sensory cell nucleus and the R which extends through the dendrite and into the soma. Scale bar 1 *µm*. *Unpublished data (Paper II).*

4 The neuronal basis of steering

4.1 The insects' internal compass is located in the centre of its brain

For an animal to successfully travel along a given bearing, it must continuously compare its current heading to its desired heading. When these headings do not align, the animal has to employ compensatory steering movements to continue its journey in the direction it aims to go. This holds true for moths migrating across Australia (Dreyer et al., 2018), ants navigating back to their nest (Wehner, 2020), and beetles transporting their balls of dung away from a dung pile (Baird et al., 2010; Dacke and el Jundi, 2018). In insects, the comparison between current and desired headings is performed in a brain region termed the *central complex* (CX) (el Jundi et al., 2018; Heinze, 2024; Honkanen et al., 2019; Mussells Pires et al., 2024; Pfeiffer and Homberg, 2014; Westeinde et al., 2024). The neuroarchitecture of this central brain region is highly conserved across all insect species studied to date, including the dung beetle *Kheper lamarcki*, and can be divided into four neuropils: the *protocerebral bridge* (PB), the *fan shaped body* (FB), the *ellipsoid body* (EB) and a pair of *noduli* (NO) (see Figure 15, (el Jundi et al., 2019, 2018; Immonen et al., 2017)). The CX is further arranged into vertical columns, connected via columnar neurons extending from the PB to columns in either the FB or the EB (el Jundi et al., 2018; Hanesch et al., 1989; Heinze et al., 2013; Heinze and Homberg, 2008; Hulse et al., 2020; Wolff and Rubin, 2018).

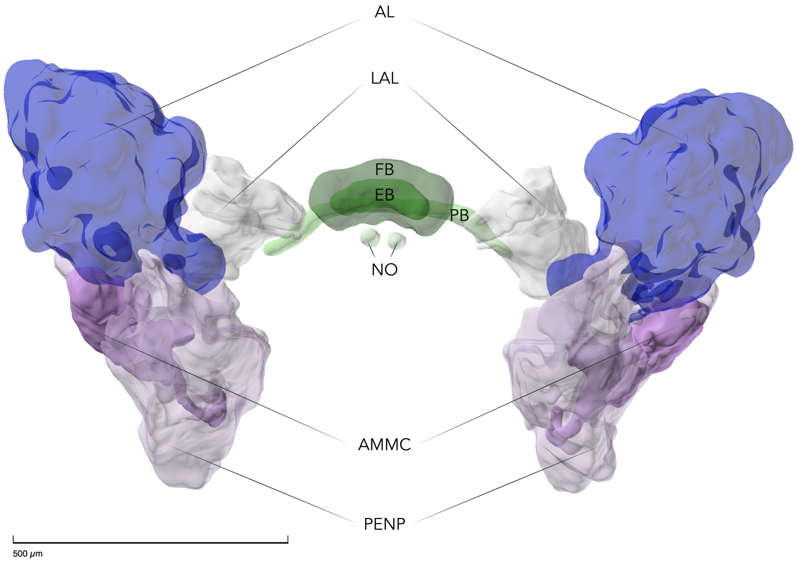

Figure 15: Volumetric rendering of neuropils in the brain of *K. lamarcki*.
Depicted are the antennal lobes (AL, blue), the lateral accessory lobes (LAL, white), the antennal mechanosensory and motor centres (AMMC, purple), the periesophageal neuropils (PENP, pale purple), and the central complex (green). The central complex is in turn divided into the fan shaped body (FB), the ellipsoid body (EB), the protocerebral bridge (PB) and the paired noduli (NO). *From (Immonen et al., 2017).*

To discern its current body orientation with respect to the surrounding space, an animal can rely on *idiothetic* (internal) or *allothetic* (external) cues. Idiothetic cues – that arise as a function of the movement of the animal itself – unavoidably accumulate errors over time. Consequently, directional information from external directional cues, whose position in space remain unaffected by the movement of the animal, becomes advantageous to support navigation over longer distances (Cheung et al., 2007; Souman et al., 2009). The accumulation of errors in the absence of external direction information can clearly be observed in *K. lamarcki* when striving to move its ball of dung along a consistent bearing in the dark; in the absence of visual cues, it soon begins to circle (Khaldy et al., 2019)

In insects, the orientation of the body relative to the external world is decoded in the *head direction circuit* of the CX. This neuronal network is composed of neurons with dendritic arborisations in the EB and PB (E-PG and P-EN neurons, respectively). Recent results from the study of the head direction circuit in *Drosophila melanogaster* reveal that directional information, from e.g. visual and mechanosensory brain regions, is relayed to the EB via *ring neurons* that encode the azimuth of available directional cues (Okubo et al., 2020; Seelig and Jayaraman, 2013). These neurons synapse onto E-PG neurons, whose subsequent responses manifests as an *activity bump* that conveys the animal's current head direction in relationship to the external world (Seelig and Jayaraman, 2015; Stone et al., 2017).

If an animal rotates, the representation of the heading direction in the EB (the activity bump) must be updated accordingly. In flies, this is made possible by the P-EN neurons (Green et al., 2017; Turner-Evans et al., 2017). The P-ENs extend from the PB to the EB and form a recurrent circuit with E-PG neurons. If the insect is moving along a straight line, the activity in P-EN neurons is equal in both hemispheres of the PB and the activity bump in the EB is stabilised in one position. If the animal then rotates, certain populations of P-EN neurons will be excited, causing an increase in activity in one hemisphere (Honkanen et al., 2019). Due to the nature of the P-EN connections to the E-PGs, this imbalance in P-EN activity across the hemispheres will excite a different subset of E-PGs and inhibit others, thus shifting the activity bump to match the new head direction in relation to the external cues (see Figure 16, (Honkanen et al., 2019)).

Neurons and neuronal structures analogous to those describe above have been identified in the brain of the dung beetle *K lamarcki* (el Jundi et al., 2018, 2015; Immonen et al., 2017), and it is reasonable to assume that rotational movements are decoded and support the beetle's representation of its head direction according to the same neuronal principles. Electrophysiological recordings from ring neurons and E-PG neurons in the beetle brain also clearly demonstrate that these cells respond to the azimuthal position of visual directional cues (el Jundi et al., 2015). Wind-sensitive cells (see ***Section 4.3***) are however yet to be identified within the dung beetle's CX.

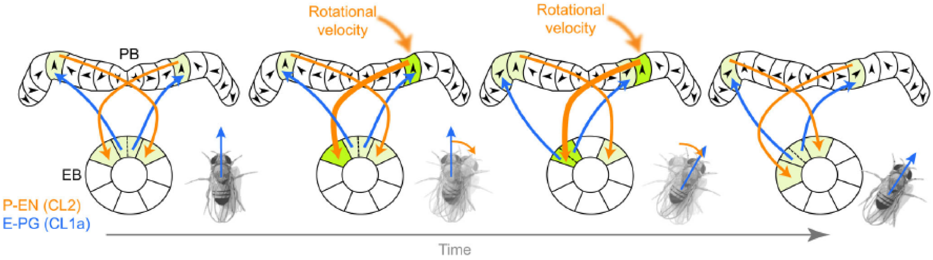

Figure 16: The head direction circuit of the fruit fly and its tracking of rotational movements. Illustrated is the activity bump (green) in the ellipsoid body (EB) and the corresponding activity in the protocerebral bridge (PB). Black arrowheads in PB columns illustrate directional preference of each column. When the fly is stationary, P-EN activity remains balanced between the brain hemispheres and the E-PG activity bump is stabilised. Rotation causes imbalanced P-EN activity, resulting in excitation (thick arrows) of certain E-PGs and inhibition (thin arrows) of others, subsequently leading to an updated representation of the head direction. *From Honkanen et al. (2019).*

4.2 Towards a definition of the wind compass pathway of the beetle *K. lamarcki*

In dung beetles, previous dye injections at the level of the antennal flagellum revealed projections from the antennal nerve into the *antennal lobe* (AL), the *antennal mechanosensory and motor centre* (AMMC), and the *gnathal ganglia* (GNG) (Immonen et al., 2017). These injections did however not aim to define whether these projections were Johnston's organ specific or if they originated from other sensors in the antennae, for example from olfactory hairs strewn across the flagellum. To define the dung beetle's wind-encoding pathway, and to discern the projection pattern of Johnston's organ neurons in the brain, I decided to perform anterograde, differential staining of the antennal nerve and Johnston's organ afferents (Box 2). This allowed me to distinguish fibres that specifically originate in the Johnston's organ and how they project into the beetle's brain.

While preliminary, my results verify that afferent neurons from the dung beetle Johnston's organ project to the antennal lobe (AL) (see Figure 17). A portion of these fibres bypass the AL and instead project into the closely associated antennal mechanosensory and motor centre AMMC (see Figure 17). From there, I observe a visible branch extending into the *periesophageal neuropils* (PENP), possibly projecting to the gnathal ganglia (GNG) (see Figure 17). These Johnston's organ projections are in line with what Immonen et al. (2017) observe in their antennal staining, as well as what has been identified in desert ants (Grob et al., 2021b), honeybees (Ai et al., 2006), and fruit flies (Kamikouchi et al., 2006). In these insects, the axons of the bipolar sensory cells of the Johnston's organ gather into distinct bundles that project alongside the antennal nerve before converging with it near the scape-pedicellar joint (Ai et al., 2006; Grob et al., 2021b; Kamikouchi et al., 2006). In parallel to what I observe in the beetles, some of these antennal nerve's afferent projections extend to the *antennal lobe*, while another portion relays information to the *antennal mechanosensory and motor centre* (AMMC, see Figure 15). Furthermore, in the desert ant, Johnston's organ fibres project to the *ventral complex*, the *ventrolateral protocerebrum*, and the *posterior slope*, which are brain regions known to receive visual input, indicating areas where information from multisensory cues converge (Grob et al., 2021b). I could not observe similar fibres, or neurons projecting in closer proximity to the central complex; whether this is rooted in species-dependent differences, or an artefact of sample preparation is unknown at this stage. To further unravel the wind-pathway of the dung beetle *K. lamarcki*, additional antennal dye injections, coupled with electrophysiology, are needed.

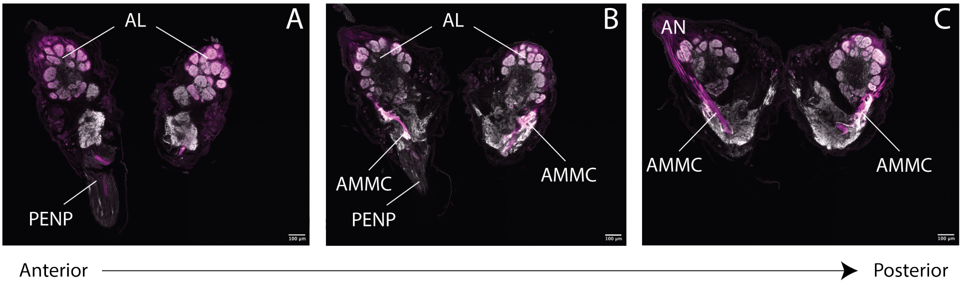

Figure 17. Optical sections through the antennal lobe in the brain of *K. lamarcki* from anterior (A) to posterior (C); stained with anti-synapsin (grey) and dye injected (magenta) at the antennal level. The fibres labelled in magenta originate in the second antennal segment, the pedicel. Antennal lobe, AL; Periesophageal neuropils, PENP, Antennal mechanosensory and motor centre, AMMC; Antennal nerve, AN. Scalebar 100 *µm*.

4.3 The pathway from the antennae to the central complex

In contrast to our current, limited understanding of the neural circuitry that underlies the processing of directional information given by wind in the beetle, this circuitry has been described in detail in the fruit fly *D. melanogaster* (Currier et al., 2020; Okubo et al., 2020; Suver et al., 2019) (see Figure 18). Here, wind induced signals elicited by antennal displacements are transmitted from the AMMC to a brain region called the *wedge*; specifically, to GABAergic neurons termed *WL-L* (one per brain hemisphere). The WL-L neurons transfer the wind-induced signal to a subset of ring neurons (termed *R1*) in the *lateral accessory lobe* (LAL) in the opposite brain hemisphere (Okubo et al., 2020). The R1 neurons, in turn, relay their wind-input to the E-PG neurons. Calcium imaging of activity in the EB of restrained fruit flies confirm that *i)* wind elicits an activity bump in the head direction circuit and *ii)* when the wind direction is shifted, the bump responds accordingly (Okubo et al., 2020).

Upon excitation of one of the WL-L neurons, Okubo et al. (2020) observe an inhibitory response in the contralateral R1 ring neurons, and excitation in the ipsilateral R1s. In other words, the R1s receive antagonistic input from the two WL-L neurons, which in turn are stimulated by displacements in the contralateral antenna, thereby bridging the circuit between antennae and head direction network. The relative ratio of inhibition and excitation imposed on each R1 by the ipsi- and contralateral WL-L neurons varies depending on antennal displacement, which in turn is dependent on wind direction. Electrophysiological recordings of R1 activity in response to wind from different directions clearly demonstrate that the bilateral integration results in R1s with individual directional tuning, i.e. a preferred wind direction that elicits greater activity compared to other wind directions. Interestingly, the R1 tuning curves proved relatively unaffected by wind strength, and Okubo et al. (2020) suggest that this is an effect of the bilateral integration. Notably, the wind-encoding pathway described in fruit flies relies on information from *both* of the insect's antennae in order to extract directionality from wind. This stands in contrast to my behavioural work presented in ***Paper I***, where I find that beetles with *one* antenna continue to perform consistent and precise wind-guided straight-line orientation. Given that in fruit flies, each WL-L carries displacement information from *one* antenna, and wind direction is given as a result of the bilateral integration of input from both WL-L neurons, it would be insightful to also identify these neurons in the beetle WL-L neurons. If found, further electrophysiological studies would be valuable to understand how their signalling of wind compares to that of the fruit fly. The decoding of wind from *one* antenna alone, as observed in beetles (***Paper I***), would possibly require alternative encoding properties or alternative pathways.

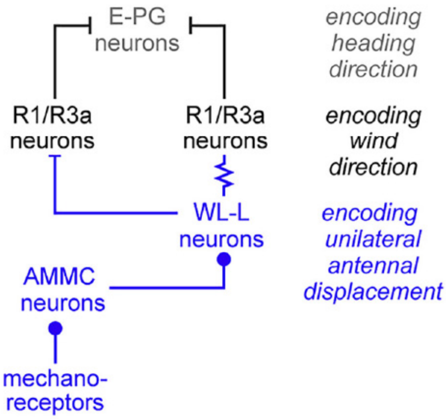

Figure 18. Schematic wiring diagram illustrating the cell types and connections involved in transducing mechanosensory information from antennal displacements to the head direction network.

The E-PG neurons are only four synapses downstream from the antennal Johnston's organ. Signals originating from mechanosensors in the antennae remain unilateral as they are relayed to the contralateral WL-L via the ipsilateral AMMC (blue connections). Wind-induced signals from each antenna do not converge until they reach the ring neurons (black connections), where bilateral integration occurs. R3a is another subset of wind-sensitive ring neuron, however their contribution to the EP-G wind response weaker compared to R1; silencing R3a had no effect on the activity bump's response to wind, whereas silencing the R1s eliminated wind EP-G wind responses. Filled circle indicate excitatory connections, the resistor symbol illustrates connections through gap junctions, and the bar represents inhibitory connections. *From Okubo et al. (2020).*

Box 2: Anterograde staining of antennal projections

To visualise the neuronal projections from the antennal Johnston's organ, I performed anterograde, differential dye injections in accordance with the protocol detailed in (Grob et al., 2021) (see Figure B2). To distinguish between projections from the Johnston's organ and the remainder of the antennae, this method relies on differential staining; (1) the antenna was cut at the first flagellomere and a green dye (Dextran Alexa Fluor 488, D22910, Life Technologies GmbH, Darmstadt, Germany) was injected, (2) the antenna was cut more proximally, at the pedicellar level, and a magenta dye (micro-Ruby, D-7162, Life Technologies GmbH, Darmstadt, Germany) was injected. As a consequence of this type of staining, all afferent projections from the antenna will be stained green, whereas those originating in the pedicel will be stained magenta. After allowing sufficient time for the dyes to be transported into the brain, the brains were dissected and put through an immunohistochemistry protocol with anti-synapsin antibodies (SYNORF1, mouse@synapsin) to visualise the neuropils and make it easier to discern into which brain region the dye-injected neurons project.

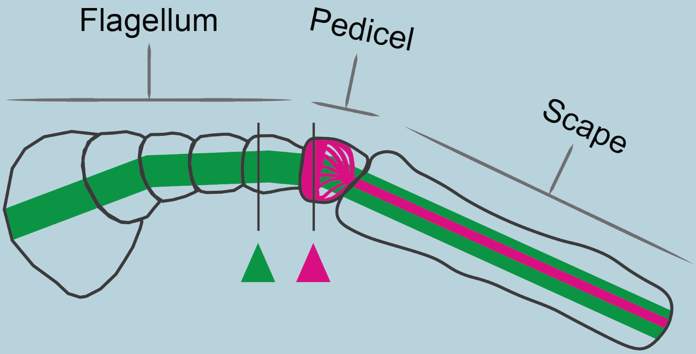

Figure B2. Schematic beetle antenna illustrating the distalmost flagellum, the pedicel (wherein the wind-sensitive Johnston's organ lies), and the scape, the most proximal antennal segment. The green and magenta triangles denote where the respective dyes were injected. The antennal nerve is labelled in green, and the Johnston's organ afferent projections are labelled magenta.

4.4 Weighted cue integration for straight-line orientation

In nature, animals often have an abundance of external directional cues at their disposal, and these multiple sources of directional information are also often combined to guide a given behaviour (Buehlmann et al., 2020b). Cue integration is a well-known phenomenon in humans (Chen et al., 2017; Ernst and Banks, 2002; Ernst and Bülthoff, 2004; Nardini et al., 2008), monkeys (Fetsch et al., 2011; Gu et al., 2008), rodents (Jeffery et al., 2016; Knight et al., 2014), and insects (Buehlmann et al., 2020b). It has, for instance, been shown that deserts ants integrate celestial and terrestrial cues (Collett, 2012; Collett and Cardé, 2014; Legge et al., 2014; Wystrach et al., 2015), as well as the directional information provided by the sun and the wind (Collett and Cardé, 2014; Müller and Wehner, 2007; Wehner et al., 2016), to find their way home. Long distance migrators, such as bogong moths and monarch butterflies rather rely on combinations of the earth's magnetic field, the visual panorama, and celestial cues to guide their impressive journeys (Dreyer et al., 2018; Franzke et al., 2020; Reppert et al., 2010). Not surprisingly, integration of directional information from the sun, other skylight cues and wind has also repeatedly been demonstrated to support straight-line orientation (Dacke et al., 2019; Khaldy et al., 2022); ***Paper III***). The underlying integration mechanisms at play are however harder to define.

An integration mechanism that is frequently presented in the literature is the '*winner-take-all (WTA)*', in which an animal faced with multiple cues gauges the reliability of each and subsequently appoints maximum weight to the cue deemed most robust, ignoring the other. This integration strategy was previously proposed as a possible explanation for *K. lamarcki*'s apparent "switch" from a sun guided compass to a wind guided compass when the reliability of the former declined in the middle of the day (Dacke et al., 2019), but was later proven wrong by my work presented further below. Another possible mechanism is the '*weighted arithmetic mean (WAM)*' and its circular equivalent the '*weighted vector sum (WVS)*', wherein the weight given to each cue is averaged to generate a combined estimate (Murray and Morgenstern, 2010). WAM and WVS are considered *statistically optimal* integration mechanisms, meaning that they strive to minimise the variance of the combined estimate, in turn maximising its reliability (Hoinville and Wehner, 2018; Murray and Morgenstern, 2010). It is important to note that this does not necessarily mean that the variance of the combined cue is lower than that of the individual cues. When the directional information from different sources points in difference directions, integration by either of these averaging strategies can result in an intermediate path (Khaldy et al., 2022; Lebhardt and Ronacher, 2014; Legge et al., 2014; Wehner et al., 2016; Wystrach et al., 2014).

My colleagues and I were interested in expanding the work on sun and wind integration in *K. lamarcki* (***Paper III***) and to identify the integration strategy at play. To do this, we took beetles into a tightly controlled indoor setup (Box 1, page 27) and performed a series of cue conflict experiments (0°, 60° or 120° azimuthal conflict) between a simulated sun and a simulated wind. As we observed a decrease in orientation precision with increasing solar elevation (***Section 2***), we chose to conduct the experiments at solar elevations of 45°, 60°, 75° and 86°. The simulated wind was set to 2.5 *m/s*. For a schematic of the experimental flow, see Figure 19A. For detailed description of the experimental methods see ***Paper III***.

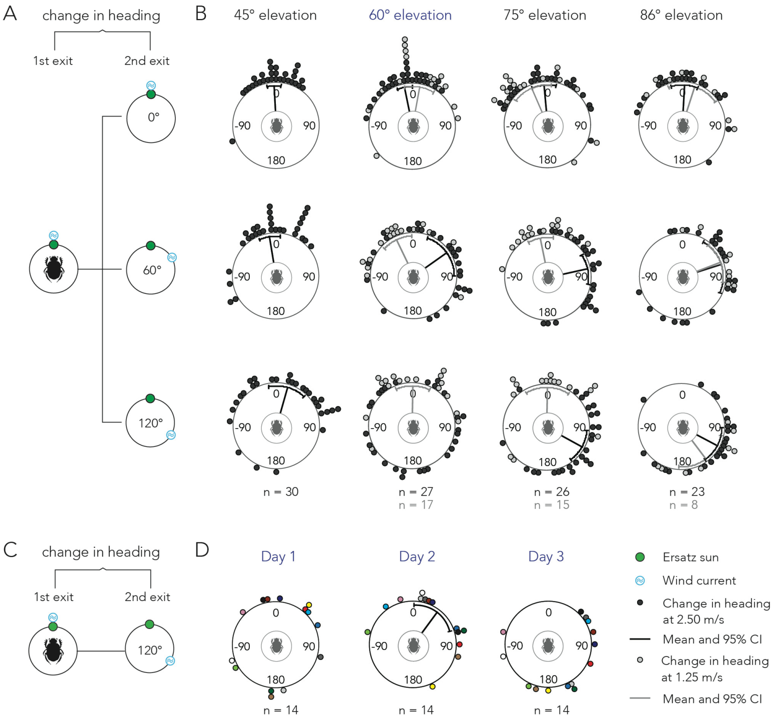

Figure 19: Behavioural results obtained in cue conflict experiments.
(A) Experimental procedure. The beetles' response to the cue conflicts was calculated as the change in heading between two consecutive exits: 1st exit with the cues aligned (initial bearing), and 2nd exit where beetles were subjected to a 0°, 60° or 120° cue conflict (test condition). (B) Changes in heading at 2.5 *m/s* wind speed (black data points) and 1.25 *m/s* (grey data points). Lines extending from the centre of the graphs indicate the population mean vector, as well as the 95% confidence interval. Elevations are indicated above the graphs. (C) Experimental procedure carried out during the three-day trials, during which the 2.5 *m/s* wind was shifted by 120° in relation to the simulated sun. (D) Changes in heading across three days. Each coloured data point indicates the response of the same individual beetle. *From Paper III*.

4.5 Dung beetles integrate sun and wind cues continuously and in a weighted manner

When the directional information of the simulated sun and simulated wind were aligned (0° cue conflict), we observed that the beetles maintained their bearings between two consecutive exits regardless of solar elevation (see Figure 19B, top row, black data points), clearly demonstrating that our setup could be used to sustain straight-line orientation. Similarly, at a 45° solar elevation, when the wind direction was shifted by 60° and 120°, the beetles continued along their initial bearings, suggesting that they were primarily relying on the simulated sun to guide their behaviour (see Figure 19B, first column, black data points), or in other words, the sun was afforded greater weight relative to the simulated wind. In contrast, at 86° and 75° solar elevations, the beetles responded to the 60° and 120° directional change of the wind by adjusting their travel direction in accordance with it, demonstrating that at these elevations, the wind, rather than the sun, was afforded greater weight (see Figure 19B, third and fourth columns, black data points). Initially, it may appear as though the beetles were simply selecting the more reliable cue over the other, which implies that they rely on the WTA integration strategy previously suggested (Dacke et al., 2019). However, in this more tightly controlled setting, we could convincingly demonstrate that the population spread increased with increasing conflict, suggesting that both cues were in fact being integrated continuously. This would not be the case in a strict WTA, but could be described by a circular, weighted integration model in which all available cues are considered when generating a combined directional reference (Murray and Morgenstern, 2010).

4.6 Orientation behaviour is variable when cue reliabilities intersect

Our results suggested that the solar elevation at which the reliability of the 2.5 *m/s* simulated wind succeeds that of the simulated sun is between 45° and 75°. At a 60° solar elevation, the beetles again responded in accordance with the 60° directional shift of the simulated wind, but – quite to our surprise – when the cue conflict was set to 120°, the beetles appeared disoriented (see Figure 19B, second column). Additional experiments revealed that beetles exposed to this 120° conflict between the sun and the wind were able to maintain their new "random" bearing consistently, and when the cues were returned to their initial, aligned position, the beetles recovered their initial bearings. This demonstrates that the beetles are not lost when the sun, at a 60° elevation, and the wind (2.5 *m/s*) are placed in a 120° conflict, and that the beetles' seemingly random bearings taken under this specific condition was an effect of their integration strategy. Similarly, the ball-rolling dung beetles

Garreta unicolor and *Garreta nitens* appear disoriented when presented with a conflict between a sun cue and polarised light, but recover their initial bearings when the cues are returned to their original positions (Chen et al., 2017; Khaldy et al., 2021). Furthermore, bogong moths presented with a conflict between the magnetic field and visual landmarks fly in a seemingly disoriented manner but, again, when the cues are returned to their original positions the moths recover their initial bearings (Dreyer et al., 2018).

My colleagues and I hypothesised that at a 60° solar elevation and a 2.5 *m/s* wind speed the reliabilities of the simulated sun and simulated wind intersect. To study this in closer detail we repeated the experimental condition (60° solar elevation, 120° conflict) with one population across three days. We found that the response to the azimuthal shift of the simulated wind varied over the days (see Figure 19C and D, first column). In a WTA, the intersecting reliabilities would result in a randomly broken tie, and we would have expected to observe two distinct responses among the beetles (one group of beetles that relied on the sun and one group that relied on the wind). This variability in population response when cue reliabilities are similar acts as further evidence that the beetles compass system does not employ a WTA, but rather a weighted integration strategy.

4.7 The weight given to a cue is determined by its relative reliability

Moving forward, we wanted to understand what cue parameter(s) that control the relative weight given to its directional information. We therefore conducted the same experimental assay as detailed above, but lowered the wind speed to 1.25 *m/s*. Interestingly, we found that the beetles now afforded greater relative weight to the simulated sun up to a 75° solar elevation (previously at this elevation the 2.5 *m/s* wind had dominated). At an 86° solar elevation, the beetles again responded to the directional shift of the simulated wind, implying that they are now weighing the wind above the sun (see Figure 19B, grey data points). This demonstrates a highly dynamic and adaptable compass system in which the relative reliability of a cue seems to determine the weight afforded to it, similar to what has previously been observed in humans (Chen et al., 2017), homing ants (Lebhardt and Ronacher, 2014; Legge et al., 2014; Wystrach et al., 2014) and dung beetles orienting to sun and polarised light (Khaldy et al., 2022).

4.8 The behavioural data is best replicated by a vector summation model with non-optimal weights and small, individual biases

To further uncover the integration strategy at play, five cue integration models were tested against each other to determine their likelihood to predict our behavioural data. These models were: winner-take-all (WTA), the linear weighted arithmetic mean (WAM) and its circular equivalent the weighted vector sum (WVS). We also included a non-optimal vector sum (NVS), which exaggerates the pseudo-WTA observed in our results. Finally, to account for the variable behaviour seen when the reliabilities of the cues intersect, we included a non-optimal vector sum with small, individual biases for either cue (BVS). We found that the BVS best replicated our behavioural data (see Figure 20), leading us to conclude that *K. lamarcki* integrates cues in a non-optimal weighted manner, with individual biases that come into play when cues are similarly reliable and thus close in weight. For in-depth details regarding the modelling and behavioural work, see ***Paper III***.

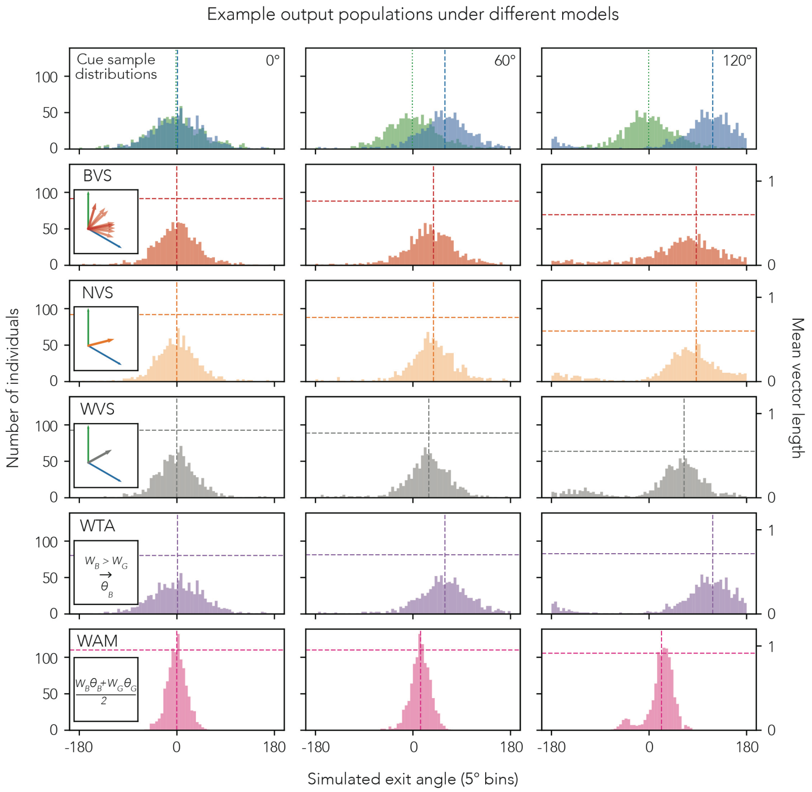

Figure 20: Output from each integration model in relation to the behavioural data. The *top row* presents two sample von Mises distributions (the circular equivalent of a normal distribution) representing the directional cues (wind – blue, sun – green); the distributions are derived from the behavioural data that defined orientation precision in the presence of the isolated cues and are used as inputs to the integration models; the dotted lines indicate cue azimuth (0°, 60°, and 120° conflict represented in the first, second, and third row respectively). In these simulations, the wind (blue) is given greater relative weight compared to the sun (green). The *remaining rows* show the estimated distributions and mean directions from each integration model when given the sample von Mises distributions; vertical dotted lines indicate mean direction of the simulated population. The mean direction generated when the sample distributions are input to the BVS (Biased Weighted Vector Sum) integration strategy best replicates our behavioural results. *From Paper III*.

4.9 The role of the antennae on the relative weight given to the directional information provided by wind

As established in ***Paper I***, dung beetles with only *one* antenna are less sensitive to the simulated wind. How does this affect the relative weight given to the directional information provided by the wind? To answer this, I decided to expose beetles with *one* antenna to a 120° directional conflict between wind at 2.5 *m/s* and a simulated sun at elevations of varying usefulness (45° and 75° solar elevations) (***Paper I***). Consistent with our findings in ***Paper III***, I observe that the relative weight afforded to the simulated wind increases as the simulated sun is shifted from 45° to 75°. However, the magnitude of this increase in relative weight varies depending on the antennal condition; at a 75° solar elevation, beetles with *one* antenna exhibit a weaker response to the 120° conflict between the sun and the wind. This demonstrates that beetles with *one* antenna afford less relative weight to the simulated wind compared to when they have *two* antennae (see Figure 21). In other words, in a scenario with multiple cues, the wind-induced directional information is relatively less useful when sensed by *one* antenna, similar to when its speed is reduced from 2.5 to 1.25 *m/s* (***Paper III***). These results further highlight the importance of the antennae for wind-guided straight-line orientation, as well as the dynamic nature of the beetle's compass system.

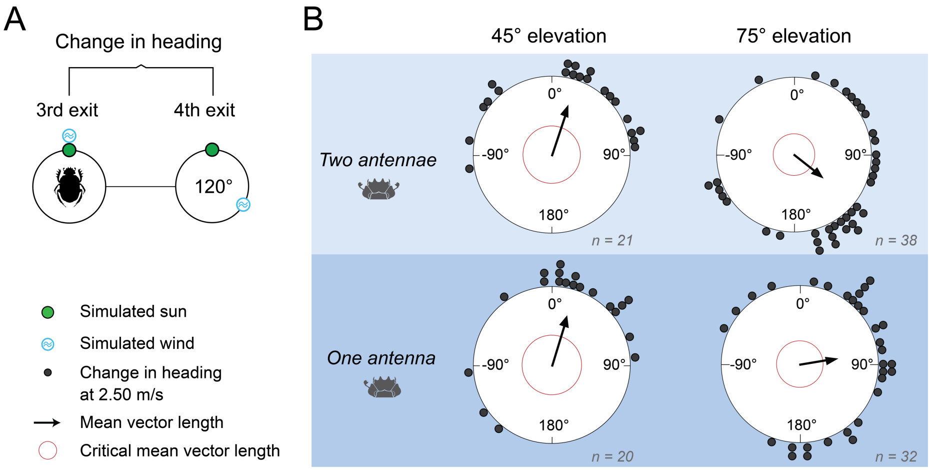

Figure 21: Changes in heading in response to a 120° cue conflict between a simulated sun and a simulated wind.

(A) Schematic depiction of the cue conflict experimental procedure. Changes in heading were quantified by calculating the difference between two consecutive exits; the 3rd overall exit, during which the simulated sun and the wind were in alignment, and the 4th overall exit, during which the directional information of the two cues was placed in a 120° conflict. (B) Changes in heading in response to the 120° cue conflict of beetle populations with *two* antennae (upper panel) and *one* antenna (lower panel), at 45° (left circular plots) and 75° (right circular plots) solar elevations. The wind was set to 2.5 *m/s* across all experiments. Each data point denotes the change in heading of an individual beetle. The arrow extending from the centre of the circular plots illustrates the population mean change in heading. The length of the arrow represents the mean vector length. The red circle represents the critical mean vector length (calculated with respect to $p \le 0.05$, Rayleigh test). *Unpublished data (Paper I)*.

4.10 Are two cues better than one?

In ***Paper III***, my colleagues and I concluded that **(1)** the reliability of a simulated sun for directional input decreases with increasing elevation, **(2)** the reliability of a simulated wind for directional input increases with increasing speed, **(3)** dung beetles integrate cues according to a weighted vector summation, and **(4)** while they afford greatest relative weight to the cue perceived to be most reliable in a moment in time, all available cues are continuously considered. The integration of directional information from multiple sources to guide a certain task is well-established within the field of behavioural control. Studies in e.g. humans (Ernst and Banks, 2002; Ernst and Bülthoff, 2004; Nardini et al., 2008), fruit flies (Chow and Frye, 2008), butterflies (Franzke et al., 2020), hawkmoths (Goyret et al., 2007), and ants (Buehlmann et al., 2020a) show that combining information from several, multimodal sources creates a robust estimate that facilitates more accurate behavioural output. Naturally, I wished to behaviourally investigate whether this also held true for the guidance of straight-line steering in the dung beetle *K. lamarcki* – in other words, *are two cues better than one?* To address this question, I brought the beetles into the behavioural setup detailed in Box 1 (page 27) and allowed them to exit the arena ten times in the presence of either (1) a simulated sun *or* a simulated wind, or (2) a simulated sun *and* a simulated wind. This was followed by ten exits where (1) a simulated sun *or* a simulated wind was *added*, or (2) a simulated sun *or* a simulated wind was *removed*. This procedure was repeated at 45° and 75° solar elevations, with the wind speed fixed at 2.5 *m/s*. The sun, at a set elevation, intensity, and azimuth serves as a relatively noise-free directional cue, whereas a wind current, with its propensity for turbulence, possesses relatively greater inherent variability.

I found that at a 45° solar elevation, the orientation precision of the beetles consistently *increased*, or remained unchanged, in the presence of a simulated sun and simulated wind (see Figure 22A). Interestingly, when increasing the solar elevation to 75°, orientation precision rather *decreased* in the presence of both cues compared to when beetles only had one cue at their disposal (see Figure 22B). My results suggest that the influence of cue noise on the behavioural output is dependent on cue weight; at a 75° solar elevation, the relative weight of the directional cues shifted towards the wind, and thus its inherent noise began to weigh more as well. These findings clearly demonstrate that drawing guidance information from multiple cues does not necessarily generate the most accurate behaviour. There are few accounts of similar results in the literature; studies on odour plume tracking in male cockroaches have shown that the addition of a visual stimulus does not result in more accurate tracking (Willis et al., 2011, 2008), and the loss of a simulated wind during odour tracking does not affect the performance of the same cockroach species (Willis et al., 2008). These results stand in contrast to similar work in ants, where the animals displayed straighter tracking paths when provided with both odour and visual cues compared to when they only had an odour cue (Buehlmann et al., 2020a).

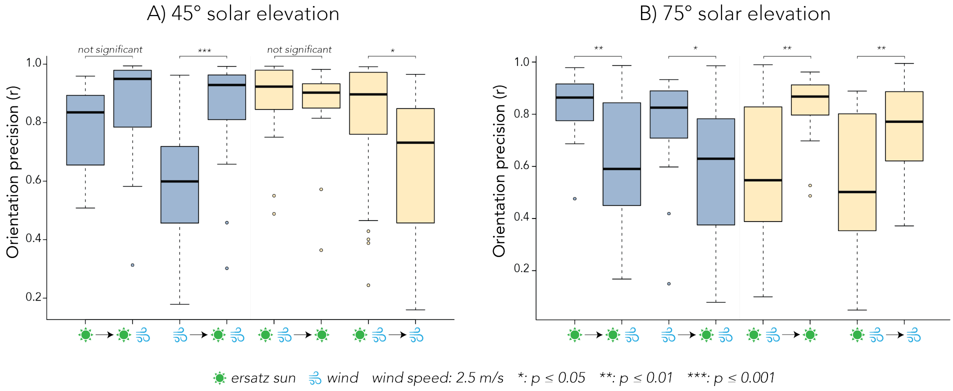

Figure 22: Orientation precision (r) in the presence of one or two directional cues.
The boxplots show the median (black line) orientation precision, interquartile range (boxes), and the minimum and maximum values (whiskers) of beetles rolling their dung balls in the presence of one (sun or wind) or two (sun and wind) directional cues, at a (A) 45° solar elevation, and (B) 75° solar elevation. *Adapted from Paper IV.*

4.11 Weight-by-reliability or weight-by-contrast?

It is important to note that while we use beetle orientation precision in the presence of isolated directional cues as a *proxy* for cue reliability (***Paper III***), my behavioural work in ***Paper IV*** indicates that this is likely a simplified view. As reported in ***Paper III***, the beetles' orientation precision in the sole presence of a simulated sun at a 45° elevation does not markedly differ from their orientation precision in the sole presence of a simulated sun at a 75° elevation – in other words, there is no difference in reliability between these two elevations of the simulated sun. Despite this, when presented in conflict with a wind at 2.5 *m/s*, the *influence* of the simulated sun on the behavioural output changes drastically when its elevation is increased from 45° to 75° (***Papers III*** and ***IV***); at a 45° solar elevation, the simulated sun is afforded greater relative weight compared to the wind, and when the elevation is increased to 75°, it is rather the wind that is afforded greater relative weight. This indicates that it is not strictly reliability that affects cue weight, and the subsequent directional influence that a directional cue poses on the combined estimate, but rather an additional property of the directional cues.

From our behavioural and modelling work in ***Paper IV***, we can conclude the beetles possibly weigh cues by *contrast*, that in many instances will also be tightly linked to reliability. As the elevation of the simulated sun increases, the difference in light intensity between the solar and antisolar hemispheres decreases; thus, the reliability of the sun (its variance of over time) does not change, but its contrast does. A wind current is, simply by its nature, a more variant directional cue compared to a fixed, simulated sun, i.e. it is less reliable relative to the light cue. However, while wind is a relatively noisier directional cue, when set to a constant speed its contrast remains unchanged. If cue weight is determined by contrast, we expect beetles to afford greater relative weight to a simulated wind at a set speed of 2.5 *m/s* when presented together with a simulated sun at a 75° elevation. However, as the beetles now give greater weight to the more variant (less reliable) wind, we also expect their orientation performance to be less precise compared to when the fixed sun is afforded more weight. Indeed, I observe both of these behavioural results in the work conducted in ***Papers III*** and ***IV***, suggesting that within the integration strategy employed by the beetles' compass system, cues are likely not weighed by reliability, but rather by contrast. The neural model presented in ***Paper IV*** combines aspects of the head direction circuitry of the fruit fly and the locust (Pisokas et al., 2020), and is demonstrated to support cue integration as a weighted vector summation – which is the integration strategy employed by the dung beetle. Using this neural model, computational simulations were run to replicate my behavioural experiments detailed above. In the simulations, the two weighting strategies ('*weight-by-reliability*' and '*weight-by-contrast*') were tested. As it turns out, the weighting strategy that best reflects my behavioural results is the latter, thus providing further evidence that dung beetles weigh cues by contrast. For additional details regarding the modelling, see ***Paper IV***.

5 The end point of my thesis

Just like *Kheper lamarcki's* straight-line journey eventually comes to an end, we now find ourselves at the end of this thesis. I can conclude that the dung beetle antennae, much like many other insects, carry the primary sensors that support wind-guided straight-line orientation, and have results suggesting that the directional wind information perceived by each antenna is summed (***Paper I***). I also find that *K. lamarcki*'s antennal Johnston's organ shares its detailed morphology with several other wind-orientating insects, indicating that it is this stretch sensitive organ that underlies wind sensing for straight-line orientation in the dung beetles as well (***Paper II***). In addition, me and my colleagues found that that in a scenario with multiple directional cues, dung beetles combine these sources of directional information according to a weighted vector summation integration strategy (***Paper III***), wherein the relative influence of each cue on the combined directional signal is given by its relative weight. In ***Paper IV*** we further propose that the parameter that determines the weight given to each directional cue is likely its contrast. In summary, this thesis provides the field of navigation with additional insights into the structure and function of the highly dynamic and adaptable insect compass system. It never failed to impress me!

My work also highlights multiple avenues for future studies of wind-guided orientation. An obvious continuation from this end point of my thesis is to conduct electrophysiological recordings from the neural circuitry underlying wind-encoding in the dung beetle. Furthermore, my work demonstrates the high accuracy with which the dung beetle can utilise wind currents to orientate itself. Considering that these insects orientate with their wind sensors close to the ground, where boundary layer winds are low and might fluctuate due to textures on the savannah, this is a remarkable feat. Moreover, the beetles do so while transporting a large dung ball that further influences the local wind flow. Therefore, in future investigations of the wind-guided straight-line orientation of dung beetles, it is also pertinent to perform velocimetry to visualise the nature of wind flow around the insect, its ball, and its tiny, tiny wind sensors.

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There is an enormous amount of people that have helped me throughout this crazy ride. To mention everyone would add a few more chapters to this book, and I quite literally do not have the time. That said, there are a few people who I cannot go without thanking (strap in, this is going to get sappy):

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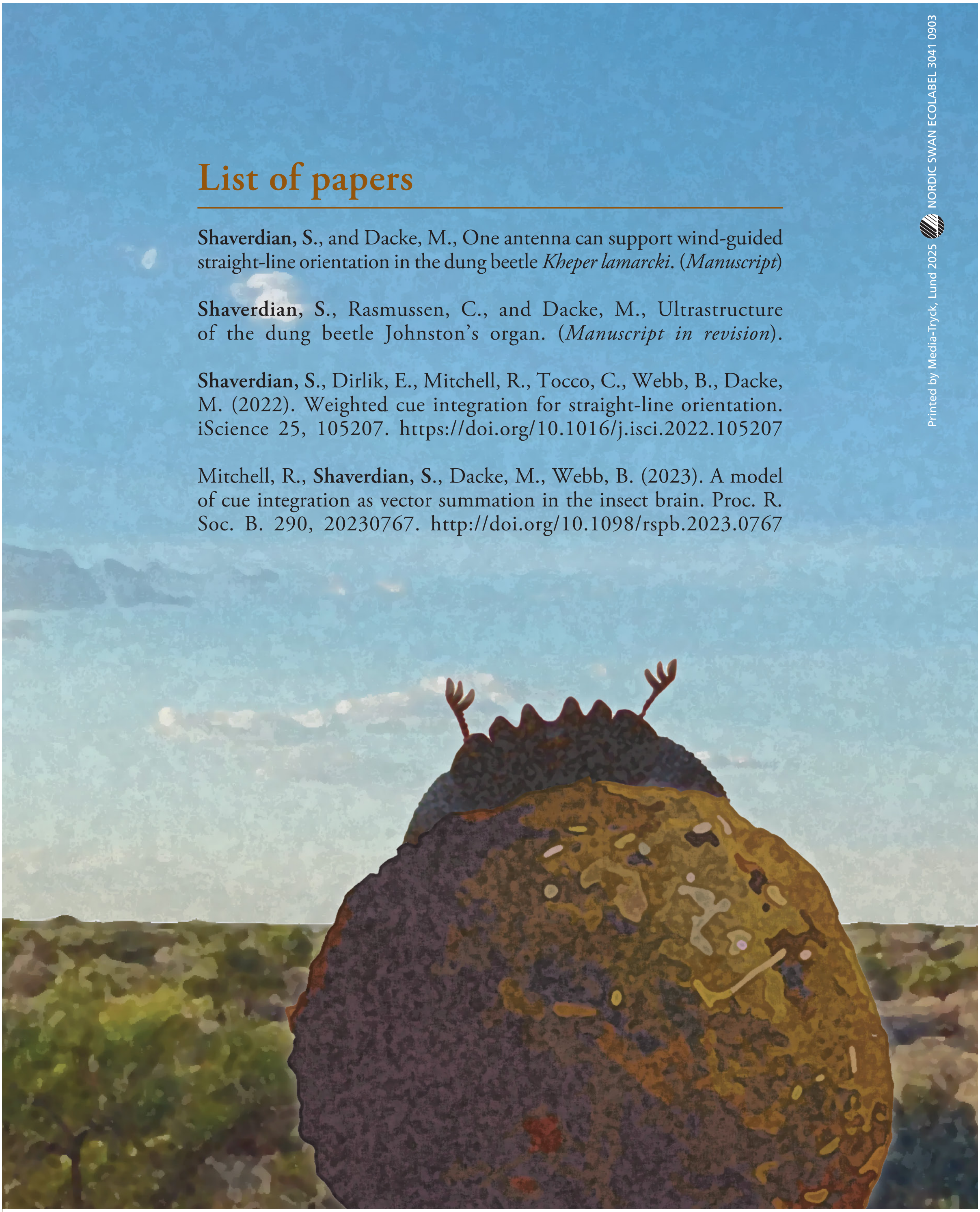

List of papers

Shaverdian, S., and Dacke, M., One antenna can support wind-guided straight-line orientation in the dung beetle *Kheper lamarcki*. (*Manuscript*)

Shaverdian, S., Rasmussen, C., and Dacke, M., Ultrastructure of the dung beetle Johnston's organ. (*Manuscript in revision*).

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