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Movements in the dark

flying, landing and walking in insects

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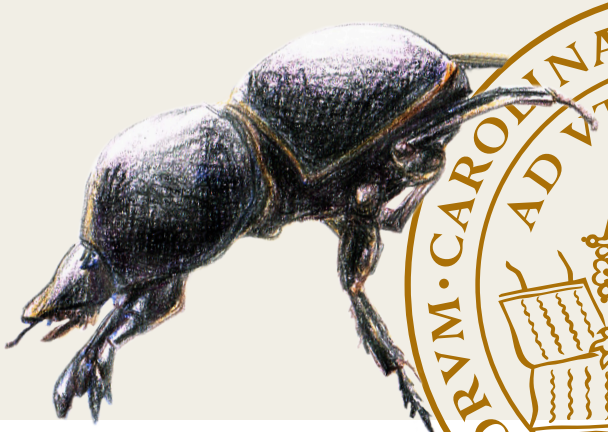
THERESE REBER
DEPARTMENT OF BIOLOGY | FACULTY OF SCIENCE | LUND UNIVERSITY 2016



Movements in the dark

Movements in the dark:
flying, landing and walking in insects

Therese Reber

LUND
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DOCTORAL DISSERTATION
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Abstract

Flying, as well as walking insects rely on vision to regulate locomotion, even in the dark when the visual system is much less reliable. To manage visual control of these behaviours at low light intensities, many insects have evolved optical adaptations, such as larger facet lenses and wider rhabdoms, and neural adaptations, such as spatial and temporal summation, to increase their visual sensitivity.

To investigate the effect of light intensity on flight control in crepuscular insects, I filmed bumblebees flying through an experimental tunnel at different light intensities. I found that bumblebees control their flight well even in dim light but fly slower as light levels fall. We also measured the effect of light intensity on the response speed of bee photoreceptors and found that they respond more slowly at lower light intensities. These results indicate that bumblebees compensate both behaviourally and visually to be able to fly in dim light.

Next, I examined the final moments of landing in bumblebees by training them to land on a flat platform that could be rotated to different orientations. I found that bumblebees adjust their body and head posture depending upon the orientation of the platform and that leg extension occurred at a constant distance from the surface (except at low platform tilts). I also investigated the effect of light intensity on the landing precision in bumblebees while landing at the same platform at two different orientations and at different light intensities. I found that bumblebees perform well-controlled landings in dim light, however, as light intensity decreased, the bees oriented their body more vertically and their head more horizontally relative to the horizontal plane and extended their legs further away from the platform. These results indicate that bumblebees rely on visual cues to perform smooth landings even in dim light.

Finally, to investigate how walking insects adapt to dim light, we analysed the orientation performance of diurnal and nocturnal dung beetles while rolling their dung balls from the centre to the periphery of a circular arena in the lab as well as in the field. We found that both species oriented well to a point light source, such as the moon or an artificial light. When only wide-field cues were present, such as starlight or the polarization pattern around the moon, the nocturnal beetles were much better oriented. Moreover, we found no effect of light intensity on ball-rolling speed, suggesting that these beetles do not employ temporal summation strategies, but rather a spatial summation approach to adapt to dim light.

To summarize, the data presented in this thesis has broadened our knowledge about insect flight, landing, walking and orientation performance in dim light and has given insights into which adaptations they might use to meet the challenges of unreliable visual signals.

Key words: Insect, Vision, Light intensity, Bumblebee, Retina, Behavioural adaptation, Flight, Landing, Dung beetle, Nocturnal adaptation, Sky compass, Straight-line orientation

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Till Caroline och Marcus

Contents

List of papers 11
Author contributions 12
Introduction 13
Vision in dim light 15
Insect eyes 17
Optical and neural adaptations 18
Flight control in insects 21
Position control 22
Flight speed control 24
Where is optic flow measured? 25
Flight control in dim light 26
Landing in insects 31
Control of deceleration 31
Leg extension 32
Landing in dim light 34
Walking in dim light 39
Conclusions 45
References 47
Att färdas i mörker: insekters lösningar, vinster och kostnader 53
Tack 55

List of papers

I. Reber T, Vähäkainu A, Baird E, Weckström M, Warrant E, Dacke M (2015) Effect of light intensity on flight control and temporal properties of photoreceptors in bumblebees. *The Journal of Experimental Biology* 218:1339-1346 DOI 10.1242/jeb.113886

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III. Reber T, Dacke M, Warrant E, Baird E (2016) Bumblebees perform well-controlled landings in dim light. *Submitted to Frontiers in Behavioral Neuroscience*

IV. Smolka J, Baird E, el Jundi B, Reber T, Byrne MJ, Dacke, M (2016) Night sky orientation with diurnal and nocturnal eyes: dim-light adaptations are critical when the moon is out of sight. *Animal Behaviour* 111:127-146 DOI 10.1016/j.anbehav.2015.10.005

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Author contributions

I. TR performed the flight control experiment and wrote the manuscript. AV performed the electrophysiological experiment and wrote the corresponding part of the manuscript. All authors participated in the design and analysis of the experiments and in the final version of the manuscript.

II. TR performed the experiment and data analysis and wrote the manuscript. All authors participated in the design and analysis of the experiment, and in the final version of the manuscript.

III. TR performed the experiment and data analysis and wrote the manuscript. MD and EB participated in the design of the experiment. All authors contributed to the data analysis and the final version of the manuscript.

IV. All authors performed and designed the experiments. JS performed the data analysis with input from BEJ, MB, EB and MD. JS wrote the manuscript. All authors contributed to the final version of the manuscript.

Introduction

Many insects rely on vision to control their position in space, to regulate their speed and to move in the desired direction. As light intensity falls, visual information becomes less reliable and, consequently, these tasks become more challenging. To meet these challenges, insects have evolved several optical and neural adaptations to increase visual sensitivity. In addition, they also employ behavioural compensation for the loss in visual resolution they often experience when moving in the dark. The aim of my thesis is to define such adaptations and to increase the current understanding for how flight, landing, and orientation performance is affected by dim light conditions.

The thesis begins with an overview of vision in dim light, where I describe the two most common eye types in insects, why it is that vision becomes less reliable as light intensity drops and what strategies insects employ to improve the sensitivity of their visual system. The next chapter continues with an overview of insect flight control, where I explain how flying insects control their position in space and how they adjust their flight speed to their surroundings. This chapter ends with a summary of insect flight control in dim light and the effect of light intensity on bumblebee flight performance. Next, I explore the landing behaviour of insects. In this chapter, I describe how flying insects control deceleration and leg extension during landing and how they land on surfaces of different orientations. This chapter also includes a summary of landing control in insects in dim light; with a special emphasis on bumblebees. In the final chapter of the thesis, I give an overview of how walking insects adapt-to and perform-in dim light conditions. The thesis ends with a conclusion, including my own contributions to the field and some ideas for future studies.

Vision in dim light

Insects have tiny eyes, but many can still function in dim light. Bumblebees, for example, are able to find flowers and forage from them early in the morning and late in the evening (Spaethe and Weidenmüller, 2002) and many nocturnal insects, such as tropical bees negotiate the cluttered rainforest at night (Warrant *et al.*, 2004). Dung beetles still find dung piles in the middle of the night, make dung balls, and use visual cues to roll them away (Smolka *et al.*, 2016).

One important task for the visual sense is to allow animals to distinguish objects that are important to them from the background, such as food or predators, but for a flying insect vision is also crucial to avoid crashing into objects during flight (*see Flight control in insects*). To discriminate an object from its background, there must be sufficient contrast between the two for the eye to detect it. In bright light, contrast discrimination is quite easy, but as light levels fall, it gradually becomes more difficult. This is partly due to the fact that fewer photons (the particles of light that are absorbed by the photoreceptors) reach the eyes in dim light and because they tend to arrive at the retina in a random and unpredictable way (Warrant and McIntyre, 1993). Pirenne (1948) explains this fact well in a famous figure (Fig. 1) in which an array of photoreceptors receives photons from an image of a dark object on a bright background. At the lowest light level, the dark object cannot be distinguished from the background because the few random photons incident on the array are only absorbed by a small number of photoreceptors. The noise (or visual unreliability) associated with these random photon arrivals is referred to as photon "shot noise". However, as light intensity gradually increases the relative level of photon shot noise decreases and the dark object gradually becomes more visible.

The effect of photon shot noise on contrast discrimination increases as light levels fall. This is because the random arrival of photons can be described by a Poisson process: the noise that is associated with the visual signal is the square root of the signal (Rose, 1942; de Vries, 1943). For example, if one photoreceptor absorbs 10 photons in dim light (a low visual signal), the noise associated with this signal will be $\sqrt{10} \approx 3.2$. If the same photoreceptor absorbs 100,000 photons in bright light (a high visual signal), the noise will be $\sqrt{100{,}000} \approx 320$. In other words, the relative magnitude of the noise is much larger in dim light, which makes the visual signal unreliable. But this is not the whole story. There are two other types of noise as well, called "transducer noise" and "dark noise". Transducer noise arises from the fact that the electrical responses that

are produced by the photoreceptors upon absorption of photons are not always the same, but vary in amplitude, duration, and latency (Lillywhite 1977; Lillywhite and Laughlin, 1979; Lillywhite, 1981; Laughlin and Lillywhite, 1982). Dark noise occurs due to the spontaneous thermal activations of the photoreceptor's transduction machinery, causing the photoreceptors to occasionally produce photon responses in complete darkness (Barlow, 1956).

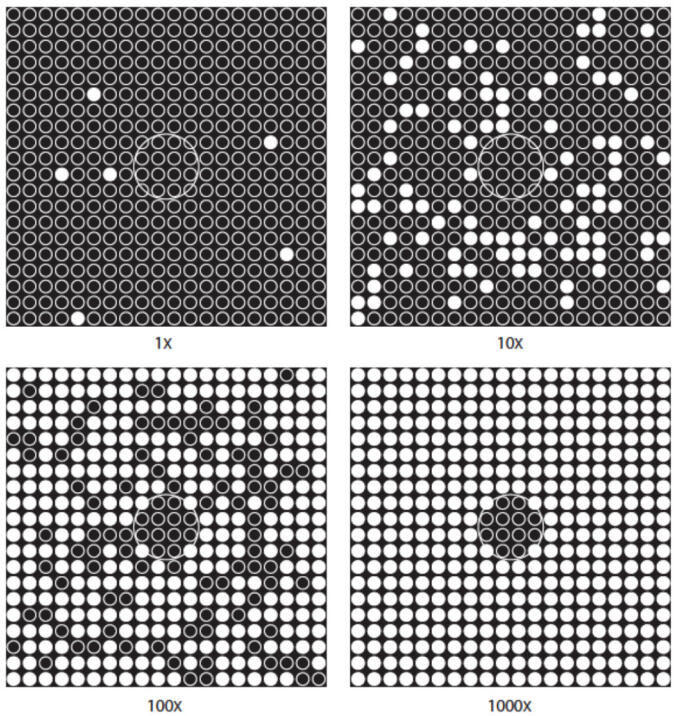


Fig. 1
The effect of photon shot noise on vision. Each square contains an array of 400 photoreceptors (small black circles) at four different light levels. The photoreceptors appear white if they have absorbed a photon. Due to photon shot noise, the black disk in the centre cannot be distinguished clearly from the background until the light level has increased 1000x. Adapted from Pirenne (1948)

Together, these three sources of noise make vision much less reliable in dim light. Still, many animals perform visual behaviours that require them to see well in dim light. To manage this, they have evolved optical and neural adaptations that increase visual sensitivity. But before discussing these adaptations, we first need to describe the morphology of insect eyes.

Insect eyes

The main organs of sight in insects are the compound eyes. Although they have the same basic function as human camera eyes, they look quite different. Instead of having one single lens as in a camera eye, compound eyes have many (typically thousands!) of small lenses, called facets. Each facet lens comprises the external surface of a single ommatidium, the basic visual unit of all compound eyes (Fig. 2) (Cronin *et al.*, 2014). The facet itself consists of an outer transparent cornea and an inner crystalline cone. Below the facet is a group of photoreceptor cells, also known as retinula cells. These cells contain visual pigments that are responsible for the absorption of the incoming light. The visual pigments are concentrated in the rhabdomeres that together make up one rod-like structure, called the rhabdom. The rhabdom can be either fused (like in bees) or open (like in flies). In each ommatidium, light is propagated through the rhabdom, with the photoreceptor cells transducing its energy into an electrical signal that is transmitted to the brain (Cronin *et al.*, 2014).

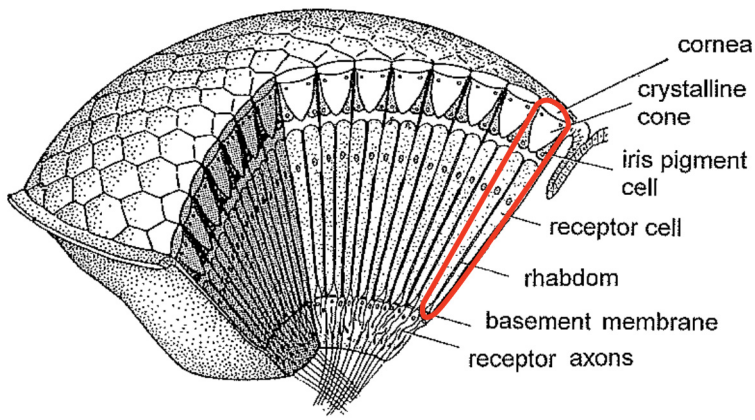


Fig. 2
Structure of an apposition compound eye with the basic visual unit, the ommatidium, surrounded by red. Modified from Land and Nilsson (2012)

There are two main types of compound eyes, which mainly differ in their sensitivity to light – apposition eyes and superposition eyes. Diurnal insect species (such as bumblebees) often have apposition compound eyes, whereas many nocturnal species (such as moths and dung beetles) more commonly possess superposition compound eyes (Fig. 3) (Land and Nilsson, 2012). In apposition eyes, each facet focuses light onto its own rhabdom. Due to the small size of each facet, each rhabdom receives only a small amount of light. On the other hand, in superposition eyes, a large number (usually hundreds, or in extreme cases, thousands) of facets focus light onto

each single rhabdom. This is possible because each crystalline cone in a superposition eye is able to refract (or reflect) the incoming light in such a way so that it leaves the cone on its way to the rhabdom as a parallel light beam. Many parallel light beams from many different cones then travel through the optically homogenous interior of the eye (referred to as the "clear zone"), finally superimposing on a single target rhabdom. These structures together make superposition eyes much more sensitive to light than apposition eyes (Cronin *et al.*, 2014). Nevertheless, not all nocturnal insects have superposition eyes. Nocturnal hymenopteran insects, such as the tropical bee *Megalopta genalis* (Warrant *et al.*, 2004; Kelber *et al.*, 2006), the Indian carpenter bee *Xylocopa tranquebarica* (Somanathan *et al.*, 2008), the polistine wasp *Apoica pallens* (Greiner, 2006), and the nocturnal ant *Myrmecia pyriformis* (Greiner *et al.*, 2007) have apposition eyes but can still perform complex visual behaviours in the dark. To manage this, their visual systems have both optical and neural adaptations for vision in dim light.

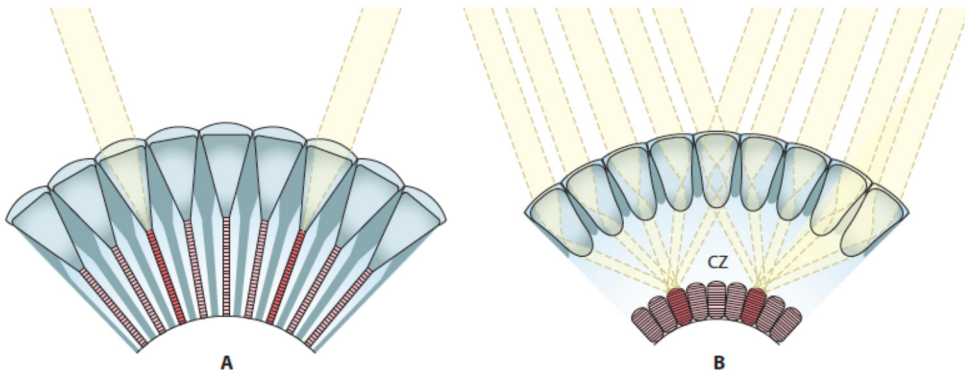


Fig. 3
The two main compound eyes. A Apposition compound eye. B Superposition compound eye. cz = clear zone. Adapted from Cronin et al (2014; Images courtesy of Dan-Eric Nilsson)

Optical and neural adaptations

The number of photons that are absorbed by the photoreceptors depends on the light collecting capacity of the peripheral structures of the eye, as well as the light absorption capacity of the photoreceptors. The capacity of the eye to collect light can be enhanced by increasing the width of the aperture, whereas the capacity of the photoreceptors to absorb light can be improved by increasing the length and width of the photoreceptors (Warrant, 1999; Greiner, 2006; Warrant, 2008; Warrant and Dacke, 2011). Many nocturnal hymenopterans, for example, have larger facets and wider rhabdoms than their diurnal relatives, such as the tropical bee *M. genalis*

(Greiner *et al.*, 2004) and the bullant *M. pyriformis* (Greiner *et al.*, 2007). However, these adaptations only increase their light sensitivity by around 27 times, which is not sufficient to explain how they are able to see at night when light levels are 8 orders of magnitude dimmer than daylight. Thus, to further increase their sensitivity to light, it has been suggested that these insects also employ neural adaptations, such as temporal and spatial summation (Warrant, 1999).

During temporal summation of the visual signals, the visual system integrates these signals over a longer period of time (Laughlin, 1990; van Hateren, 1993). This process can take place in the photoreceptors by increasing the response time, or by summing the visual signals in neural circuits higher up in the visual system (Warrant, 2004; Stöckl *et al.*, 2016). As mentioned above, bumblebees have apposition compound eyes, but are able to forage in dim light conditions (Spaethe and Weidenmüller, 2002). To examine whether they temporally sum the visual signals as light levels fall, we examined the response time of their photoreceptors at different light levels (Reber *et al.*, 2015: Paper 1). For this analysis, we studied the green-sensitive photoreceptors, known to produce the input for motion vision in bees (Kaiser, 1974; Kaiser and Liske, 1974; Srinivasan and Lehrer, 1984; Skorupski and Chittka 2010; Skorupski and Chittka, 2011). As light levels fell, the photoreceptor response time significantly increased (Fig. 4), suggesting that bumblebees have evolved visual adaptations to allow them to fly in dim light.

Temporal summation, either in the photoreceptors or in higher neural circuits, results in a much brighter image but, unfortunately, it comes at a cost: fast-moving objects will become much harder to see. This can be illustrated by comparing the integration time in the eyes to the exposure time in a camera. If an object moves across the scene, a longer exposure time may result in an image of the object that is smeared. For a fast-moving animal, such as a flying insect, this situation could be detrimental because obstacles become harder to detect or might not be detected in time, causing the insect to crash. One way to compensate for this is to move more slowly. This allows a larger amount of light to be collected per unit distance travelled. Indeed, bumblebees reduce their flight speed as light levels fall (Reber *et al.*, 2015: Paper 1). This will be discussed in the next chapter (*see Flight control in dim light*).

For spatial summation, the visual system instead sums photons in space. As light levels fall, specialised neurons with lateral branches couple neighbouring ommatidia into groups (Greiner *et al.*, 2004). This effectively increases the receptive field so that more photons can be collected. However, the spatial resolution will be compromised and, even though the image will become brighter, it will also become coarser. However, to see a blurry image is certainly better than not seeing anything at all.

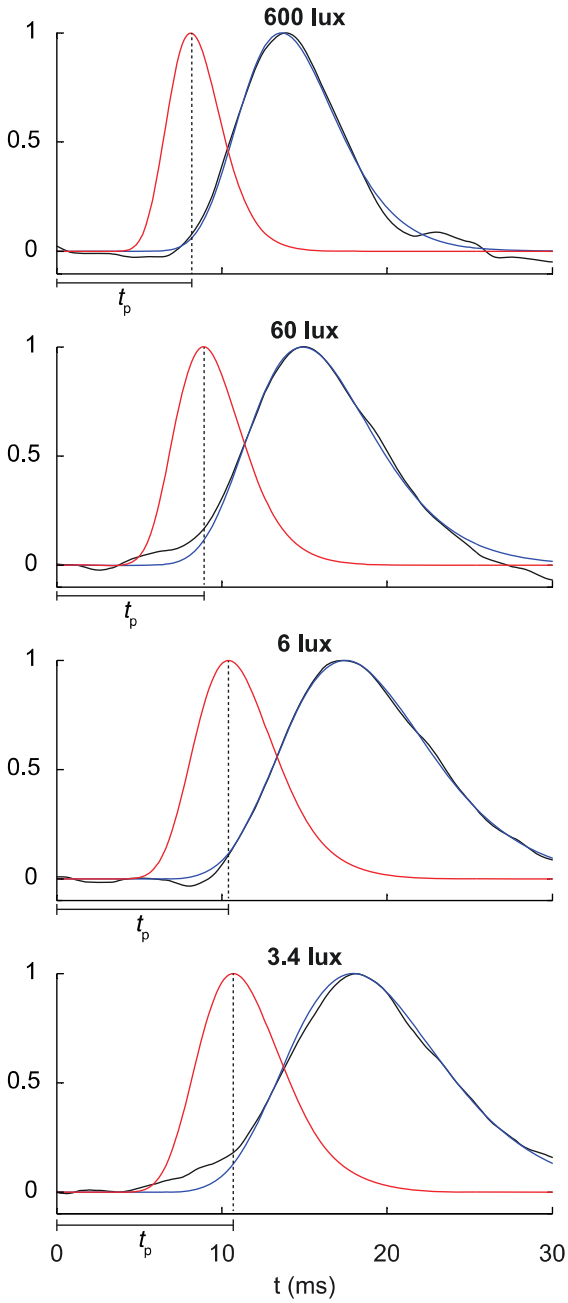


Fig. 4
The effect of light intensity on photoreceptor impulse responses in bumblebees. Black lines indicate the mean of recorded impulse responses at room temperature. Blue lines indicate the log-normal fit to the mean response. Red lines indicate the log-normal fit with parameter values temperature corrected to a flight temperature of 34.9 °C. Dashed black lines indicate temperature corrected time to peak (t_p). As light levels fall, t_p increases, i.e. the response time of the photoreceptors increases. Modified from Reber et al (2015)

Flight control in insects

Many flying insects, such as honeybees (Kirchner and Srinivasan, 1989; Srinivasan *et al.*, 1991; Srinivasan *et al.*, 1996; Baird *et al.*, 2005; Baird *et al.*, 2006; Barron and Srinivasan, 2006; Portelli *et al.*, 2010), bumblebees (Baird *et al.*, 2010; Dyhr and Higgins, 2010; Baird *et al.*, 2011; Linander *et al.*, 2015; Reber *et al.*, 2015 (Paper 1); Linander *et al.*, 2016), flies (David, 1982; Fry *et al.*, 2009; Kern *et al.*, 2012) and sweat bees (Baird *et al.*, 2011) rely on vision to control their flight. During flight, the relative motion between the insect and its surroundings generates a flow field or pattern of motion in their eyes termed "optic flow" (Gibson, 1950). Since the rate of the flow field over its eyes is related to both the speed at which the insect is flying and its distance to nearby obstacles, it contains information about the insect's own movement relative to these obstacles. In fact, the rate at which the world appears to move is inversely proportional to the distance to the objects. Thus, the shorter the distance between the insect and an object, the faster the object will appear to move across the eye. The optic flow field that an insect experiences when translating along one of the axes of freedom (vertical, lateral or longitudinal) is referred to as *translational* optic flow. A *rotational* optic flow field may also arise when the insect rotates around any of its axes, such as when the insect is turning to the left or to the right (along its yaw-axis), when it turns up or down (along its pitch-axis) or when it rolls around its body axis (roll-axis). The structure of the optic flow field can be visualized using vectors of different size and orientation (Fig. 5).

Flying insects use information from the optic flow field to control their flight in many different ways (for a review, see Srinivasan and Zhang, 1999), such as monitoring their position in space (*see Position control*) and adjusting their flight speed to the

environment (*see Flight speed control*). Optic flow information also allows insects to perform smooth landings (*see Landing in insects*).

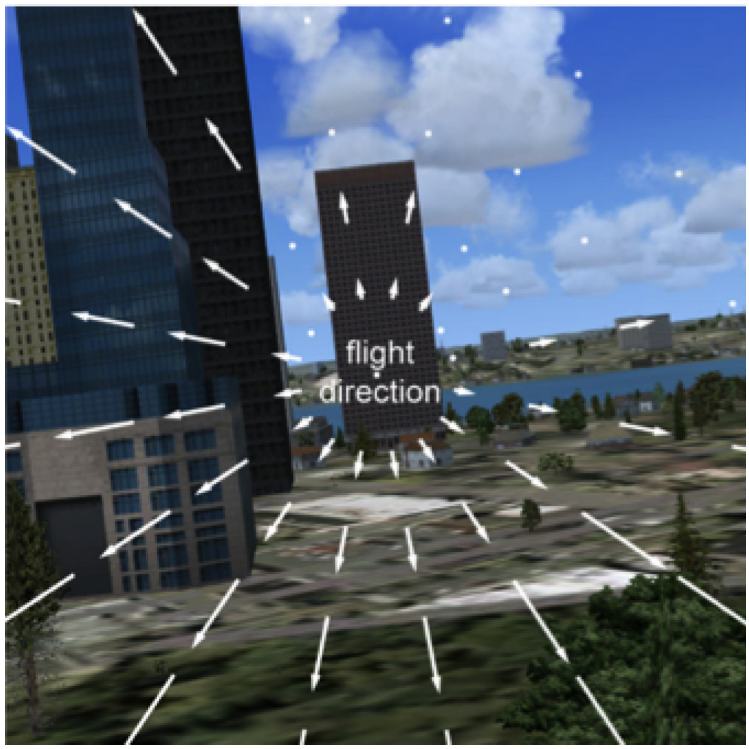


Fig. 5
Optic flow field. The length of the translational optic flow vectors indicate the relative speed of the objects in the visual field of the moving insect, whereas the direction of the vectors indicates the direction of their movement across the visual field. Objects that are far away appear to move more slowly (short arrows), whereas objects that are close appear to move more quickly (long arrows). Moreover, objects in the direction of translation do not generate flow, because there is no relative motion between them and the eyes of the insect. Image courtesy of Antoine Beyeler

Position control

Insects often fly through narrow spaces, such as between the branches in a forest or between the leaves in a patch of flowers. To avoid collisions with objects in their surroundings, bees "centre" between them by balancing the rate of the optic flow they perceive in each eye (honeybees: Kirchner and Srinivasan, 1989; Srinivasan *et al.*, 1991; bumblebees: Dyhr and Higgins, 2010; Baird *et al.*, 2011; Linander *et al.*, 2015; sweat bees: Baird *et al.*, 2011). This so called "centring response" has been demonstrated by training the bees to fly through narrow experimental tunnels lined with high contrast patterns. When a bee flies through such a tunnel, the patterns on

both walls will generate a pattern of front-to-back translational optic flow in its eyes. If both walls are lined with the same pattern (Fig. 6a), the bee will fly along the centre of the tunnel. However, if the visual patterns on the two walls are different (Fig. 6b), for example if one wall is lined with horizontal stripes (creating a weak translational optic flow field) and the other wall is lined with a chequerboard pattern (creating a strong translational optic flow field), the bee will fly closer to the wall that is lined with stripes in an attempt to balance the rate of image motion over both eyes.

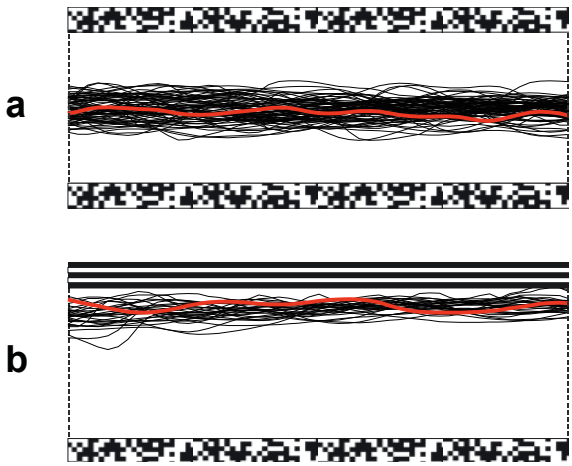


Fig. 6
Flight trajectories of bumblebees flying through en experimental tunnel (30 cm wide, 300 cm long, seen from above) with (a) chequerboard patterns on both walls, and (b) chequerboard pattern on one wall and horizontal stripes on the other wall. A typical flight trajectory for each condition is marked in red. Modified from Linander et al (2015)

The centring response in honeybees is primarily sensitive to the angular speed of the optic flow, regardless of the spatial period (the width of the stripes), the intensity profile (whether the gratings are square-wave or sinusoidal-shaped) or the contrast of the patterns (Srinivasan *et al.*, 1991). Thus, a bee that encounters a narrow passage, for example a gap between two tree stems, will centre between them, regardless of possible differences in the texture of their bark. However, a recent study on bumblebees shows that the spatial period of the visual stimuli can indeed play a role for the centring response (Dyhr and Higgins, 2010). In this study, where both walls of the tunnel were instead lined with sinusoidal vertical gratings of different spatial frequency, the bees were observed to fly closer to the wall with the highest spatial frequency (if the spatial frequency varied by a factor of at least 2.5). Since the honeybees in the earlier study were only tested within a narrow range of spatial frequencies, it is likely that also they would respond to similar differences in the spatial period of this type of stimuli.

Interestingly, when honeybees are trained to fly through a tunnel that is 95 cm wide (rather than the 12 cm wide tunnel used before), they seem to adopt a strategy that is different from centring (Serres *et al.*, 2008). If the bees enter the tunnel closer to one of the walls and if the feeder is placed close to the same wall, the bees tend to follow the wall instead, although the patterns on both walls are the same. This study suggests that bees do not always balance the rate of the optic flow between the two sides of a narrow passage, but instead strive to maintain the rate of the optic flow from one wall at a set value.

For bees that fly quickly through the dense undergrowth of rainforests, it is crucial to negotiate or avoid obstacles in an efficient way. Male orchid bees (*Euglossa imperialis*), for example, forage for nectar at high speed in tropical rainforests, while constantly avoiding collisions and negotiating gaps in the vegetation (Baird and Dacke, 2016). When trained to fly through holes of different shapes, they tend to fly close to the safest point of the apertures, i.e. as far away from the edges as possible (Baird and Dacke, 2016). To do this, they locate and fly towards the brightest spot of the aperture. Furthermore, they also rely on brightness cues to locate gaps that are large enough to fly through.

Flight speed control

Flying insects also adjust their speed according to the environment in which they are flying. In open environments with few obstacles, it is relatively safe to fly at an increased speed with little risk of collision. However, when the environment becomes more cluttered, such as in a forest, the risk of collision is higher so it is necessary to reduce flight speed to have time to react to obstacles in the flight path. To control their flight speed, flying insects aim to keep the rate of the translational (front-to-back) optic flow that they perceive constant. This has been shown in several different insects, such as flies (David, 1982; Fry *et al.*, 2009; Kern *et al.*, 2012), honeybees (Srinivasan *et al.*, 1996; Baird *et al.*, 2005; Baird *et al.*, 2006; Barron and Srinivasan, 2006; Portelli *et al.*, 2010), and bumblebees (Baird *et al.*, 2010; Baird *et al.*, 2011; Linander *et al.*, 2015) by allowing them to fly in similar types of experimental tunnels as described above.

Honeybees, for example, were trained to fly through a tapered tunnel with vertical black and white gratings, where the width of the tunnel was narrow in the middle and wide at both ends. As they reached the narrow section of the tunnel, they slowed down, and then increased their speed when the tunnel grew wider again (Srinivasan *et al.*, 1996). The flight speeds recorded in the different parts of the tunnel supported the theory that the bees strived to keep the angular image velocity of the walls constant. Furthermore, if honeybees are trained to fly through a tunnel of constant

width, where the spatial frequency of the vertical gratings changes halfway, the flight speed of the bees remain the same throughout the length of the tunnel, suggesting that flight speed control is not sensitive to spatial frequency (Srinivasan *et al.*, 1996). These results have also been confirmed in an experiment, in which honeybees were allowed to fly through a tunnel where the motion, contrast and texture of the patterns that lined the walls could be changed (Baird *et al.*, 2005). In this study, the bees also adjusted their flight speed according to the perceived rate of translational optic flow and independently of the spatial frequency, as well as the contrast of the gratings on the walls. In contrast, bumblebees that are trained to fly through a tunnel, where one wall is lined with vertical gratings of different spatial frequency, increase their flight speed when the spatial frequency is increased (Dyhr and Higgins, 2010). However, the difference in behaviour between honeybees and bumblebees might again be explained by the fact that honeybees have only been tested with a narrow range of spatial frequencies.

Another situation that increases the risk of collisions is when light levels fall. As a response to this "high risk scenario", several insects reduce their flight speed as light levels fall (honeybees: Rose and Menzel, 1981; hornets: Spiewok and Schmolz, 2005; bumblebees: Reber *et al.*, 2015: Paper 1) (*see Flight control in dim light*). Similarly, when a bee approaches a flower, it reduces its flight speed by keeping the angular velocity of the expanding image of the flower constant (Baird *et al.*, 2013). In this way, the bee will automatically slow down, reaching a speed that is close to zero just before touchdown, ensuring that it will land safely on the flower without crashing. We will return to the landing behaviour of insects in the next chapter (*Landing in insects*).

Where is optic flow measured?

An insect that flies from an open space into a more cluttered environment will experience a change in the magnitude of optic flow. To avoid collisions, it is important that the insect detects the change in time, preferably well before it approaches the obstacles. The magnitude of optic flow is not the same everywhere in the visual field of the flying insect. If the insect is flying forwards at a constant speed, it experiences a larger degree of optic flow at an angle of 90° from its direction of flight (Gibson, 1950). Thus, it is likely that changes are easier to detect if they occur in the lateral visual field of the insect. Indeed, there is some indication that honeybees adjust their position in space by measuring optic flow in their lateral visual field (Srinivasan *et al.*, 1991). However, if the insect only measures optic flow in its lateral visual field, it would only detect a cluttered environment once it had already entered it. A better strategy would be to measure optic flow in its frontal visual field at a low

viewing angle. Indeed, bumblebees that are trained to fly through a tunnel, where the distance between the walls changes halfway, respond to the change at a minimum viewing angle of about 30° from the direction of flight (Baird *et al.*, 2010). In other words, they measure optic flow at a relatively low visual angle in their frontal visual field. Honeybees have also been shown to measure optic flow in their ventral and dorsal visual fields (Portelli *et al.*, 2011). However, in these two studies it was not clear at what viewing angle the bees were measuring optic flow.

To investigate the effect of abrupt optic flow changes on the centring performance, the flight trajectories of bumblebees flying through a tunnel where the magnitude of optic flow changed on only one side of the tunnel were recorded (Linander *et al.*, 2015). This study revealed that bumblebees measure optic flow flexibly within their field of view. More specifically, they seem to extract information from the part of the visual field where the maximum magnitude of optic flow occurs. This was further investigated by allowing the bees to fly in a tunnel where the width of the tunnel could be changed by a large extent (ranging from 60 cm wide to 240 cm wide, in 60 cm steps) (Linander *et al.*, 2016). As the width of the tunnel increased, the precision of the centring response decreased and the effect of the optic flow generated by the walls on flight speed decreased. To control flight speed, the bees instead appeared to rely on optic flow from the ground, since this part of the tunnel occupied a larger region of the visual field than the walls as tunnel width increased. This result supports the finding that bumblebees measure optic flow depending on where in the visual field the largest magnitude occurs (Linander *et al.*, 2015).

Flight control in dim light

Most studies on flight control in insects have been performed in bright light. However, there are a few studies investigating the effect of light intensity on insect flight performance. These include studies on honeybees (Rose and Menzel, 1981), hornets (Spiewok and Schmolz, 2005), sweat bees (Theobald *et al.*, 2007; Baird *et al.*, 2011; Baird *et al.*, 2015), and bumblebees (Reber *et al.*, 2015: Paper 1).

In a colour discrimination experiment, honeybees were observed to decrease their flight speed when light levels dropped below 100 cd/m^2 (Rose and Menzel, 1981). At 1 cd/m^2, they often hovered in the air, crashed, and had problems landing on the experimental setup, suggesting that the flight control system of the bees suffers in dim light. Similarly, hornet workers that are tethered to a roundabout setup and induced to fly, significantly reduce their flight speed when light levels fall (Spiewok and Schmolz, 2005). The two nocturnal sweat bees (*M. genalis* and *M. ecuadoria*) forage early in the morning and late at night when the light level is extremely low (Warrant *et al.*, 2004; Kelber *et al.*, 2006). Video-recordings of return-flights to the nest

indicate that also the flight performance of these nocturnal bees is compromised in dim light (Theobald *et al.*, 2007). As light levels decreased, the average time to land at the nest entrance increased. This, however, did not depend on a lower approach speed, but rather on longer and more meandering flight trajectories. Interestingly, some bees still landed quite quickly at the nest entrance in dim light. However, these landings were associated with more errors and the bees sometimes made several attempts to land. Why did these bees not slow down like hornets and honeybees do? This is currently not known, but to fly slowly is not a trivial task and requires that the insect is sensitive to low velocity motion (O'Carroll *et al.*, 1996).

To investigate whether the nocturnal bee *M. genalis* relies on optic flow information to control their flight in dim light, just like diurnal insects do, they were trained to fly through a narrow tunnel in their natural habitat in Panama (Baird *et al.*, 2011). The tunnel was lined with different patterns providing the bees with different amounts of optic flow information. The bees were found to adjust their flight speed in the same way as diurnal insects: when the horizontal motion cues were reduced, the bees increased their speed. Like diurnal insects, they centre along the tunnel's midline when the amount of optic flow is the same on both walls. In contrast to diurnal insects, however, they still centre when the amount of optic flow is different on the two walls, suggesting that *M. genalis* extracts information from the optic flow field in a different way compared to diurnal insects. Nevertheless, the fact that *M. genalis* relies on visual information at all is remarkable given that the light intensities at which this bee is active are extremely low. As mentioned before, *M. genalis* has evolved optical adaptations to increase their sensitivity and it has been suggested that they also employ neural adaptations, such as spatial and temporal summation. However, if they sum visual information over time (temporal summation), their ability to detect image motion would decrease.

To explore whether *M. genalis* performs temporal summation, the effect of light intensity on their flight speed and their centring performance was tested while flying through the same tunnel as above at light levels ranging from 40.4 lx down to 0.0014 lx (Baird *et al.*, 2015). Despite this change in light intensity, the bees did not reduce their speed and there was no effect on their centring behaviour (although the variance within the flights suggested that there is a weak effect of light intensity on the centring response), indicating that *M. genalis* does not perform temporal summation in dim light, at least not to a significant extent.

To examine whether free flying diurnal or crepuscular insects adapt to dim light conditions with a reduction in flight speed (like honeybees and tethered hornets) or rather compromise their flight performance and fly along more winding tracks (like nocturnal sweat bees), I trained bumblebees (*Bombus terrestris*) to fly through an experimental tunnel at light intensities ranging from 600 lx down to 3.4 lx (Reber *et al.*, 2015: Paper 1). The walls and the floor were lined with random chequerboard

patterns to provide the bees with optic flow information. I found that, similar to honeybees and hornets, bumblebees decrease their flight speed as light levels fall (Fig. 7), probably to compensate for the increased visual blur caused by the longer integration times in their photoreceptors (*see Optical and neural adaptations*).

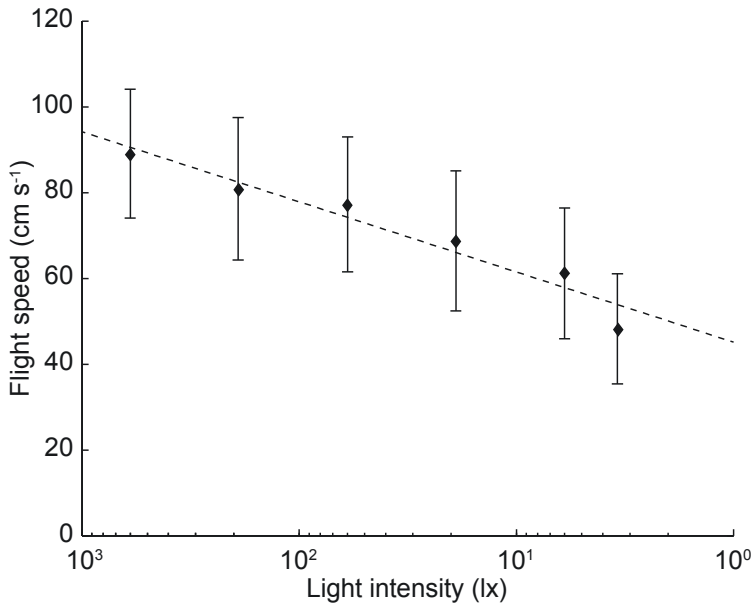


Fig. 7
The effect of light intensity on bumblebee flight speed. The symbols indicate mean values of flight speed and error bars indicate ±2 s.d. The dashed line indicates the linear correlation between flight speed and log light intensity. For more details, see Reber et al (2015)

To investigate whether the retinal and behavioural adaptations that we observed in the bumblebees allowed them to fly with the same precision through the tunnel in dim light as in bright light, I also analysed the average distance of the bees from the midline of the tunnel and the length of the flight trajectories. As light intensity fell, the centring precision decreased and the flight trajectories became more tortuous, suggesting that the bees have more difficulty in controlling their flight position under these conditions (Fig. 8). However, the bees still centred remarkably well along the midline of the tunnel, indicating that they still extract enough visual information to support a safe flight down the tunnel.

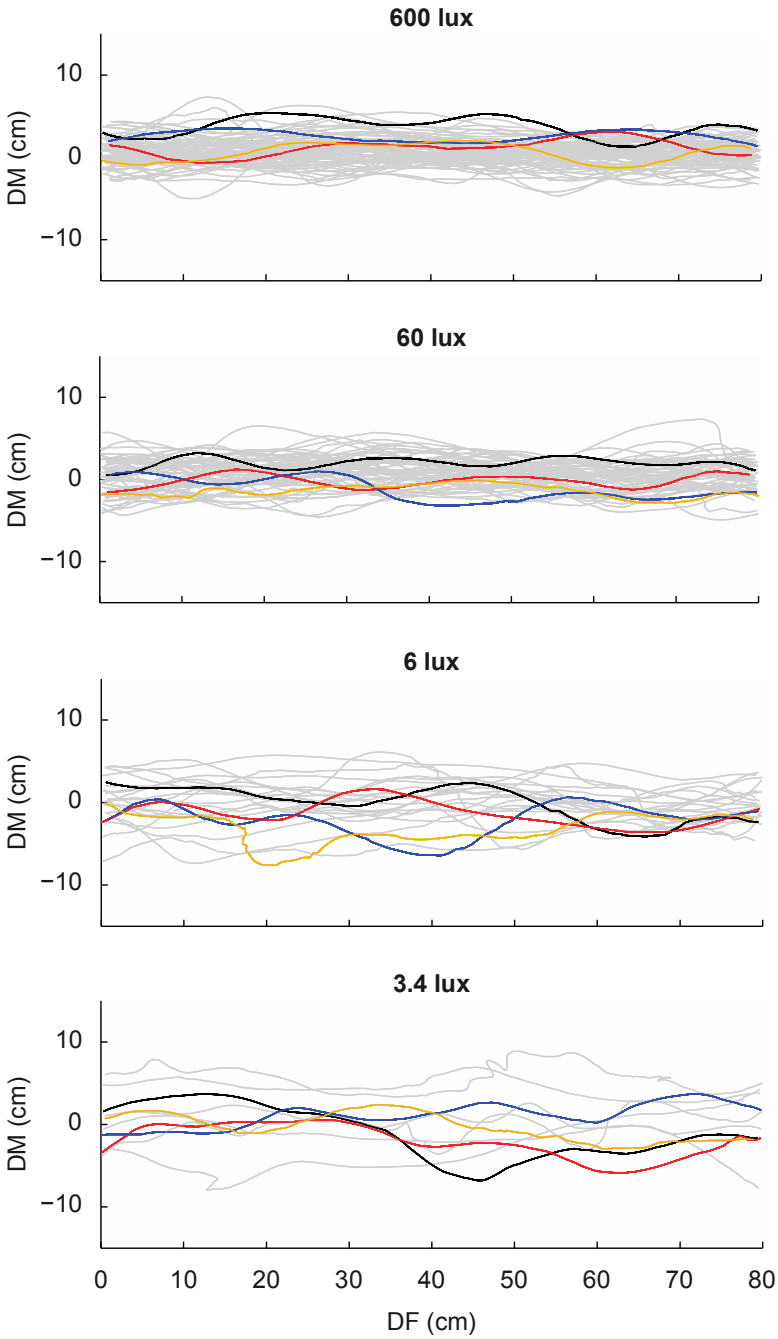


Fig. 8
Flight trajectories of bumblebees flying through an experimental tunnel (30 cm wide, 200 cm long, seen from above) at four different light intensities (600 – 3.4 lx). Four bees flew in all four conditions (highlighted in black, blue, red, and orange). DM = distance from the midline, DF = distance flown. For more details, see Reber et al (2015)

Landing in insects

As we have seen, flying insects have evolved simple and efficient strategies to control their flight speed and their position in space, but how do they control landing? A pollinating insect, such as a bumblebee worker may land on thousands of flowers over the course of a single day (Abrol, 2011). When she has filled her honey stomach sufficiently and collected enough pollen, she returns back to her nest, lands at the entrance and unloads her forage. To ensure safe and smooth landings, she has to slow down, extend her legs at the right moment in time, and adjust her body posture to the orientation of the landing surface.

Control of deceleration

To regulate deceleration during the approach to a surface, insects rely on visual motion cues (Wagner, 1982; Srinivasan *et al.*, 2000; Breugel and Dickinson, 2012; Baird *et al.*, 2013). When free flying houseflies (*Musca domestica* L.), for example, approach a landing site, they begin to decelerate when the ratio of the image size to the rate of expansion of the landing surface reaches a threshold value (Wagner, 1982). Similarly, fruit flies (*Drosophila melanogaster*) start to reduce their speed in preparation for landing when the size of the landing target and its retinal expansion rate reach critical values (Breugel and Dickinson, 2012).

Free flying honeybees also rely on visual cues to reduce flight speed during landing (Srinivasan *et al.*, 2000; Baird *et al.*, 2013). By training them to land on a flat

horizontal surface, Srinivasan *et al.* (2000) found that the bees reduced their flight speed by keeping the angular velocity of the landing surface constant. In a later experiment, honeybees were filmed as they landed on a vertical surface (Baird *et al.*, 2013). At this landing surface orientation, the bees instead regulated their approach speed by keeping the rate of the image expansion generated by the landing surface constant. This strategy is not different from the strategy proposed by Srinivasan *et al.* (2000) but is more universal and may theoretically be used for any surface orientation. Both of these strategies ensure that the speed is close to zero at touchdown.

Leg extension

To reduce drag during flight, the legs of an insect are normally folded under the body (Goodman, 1960). To ensure a safe touchdown during landing, the legs must be lowered before making contact with the surface. Leg extension has been studied in several different insects, such as flies (Goodman, 1960; Borst, 1986; Tammero and Dickinson, 2002; Breugel and Dickinson, 2012), honeybees (Evangelista *et al.*, 2009), sweat bees (Baird *et al.*, 2015), and bumblebees (Baird *et al.*, 2015; Reber *et al.*, 2016a: Paper 2; Reber *et al.*, 2016b: Paper 3).

Tethered common green bottle flies (*Lucilia sericata*) (Goodman, 1960) and tethered houseflies (*Musca domestica*) (Borst, 1986), for example, lower their legs in response to visual stimuli. The houseflies were shown to extend their legs when subject to an expanding pattern in their frontal visual field (Borst, 1986). This was also shown in tethered (Tammero and Dickinson, 2002), as well as free flying (Breugel and Dickinson, 2012) fruit flies (*Drosophila melanogaster*). More exactly, fruit flies extend their legs when the landing surface subtends more than 60° of their frontal visual field (Breugel and Dickinson, 2012). If the fruit flies instead experience image expansion in their lateral visual field, they tend to steer away from the object, presumably to avoid a collision (Tammero and Dickinson, 2002). Free flying fruit flies also sometimes steer away from the landing target (Breugel and Dickinson, 2012). In this case, the reaction tends to occur when the target reaches a threshold of about 33°.

Free flying honeybees might also rely on visual input to estimate the distance at which they extend their legs (Evangelista *et al.*, 2009). When trained to land on a flat platform that can be rotated to different orientations, the bees tend to extend their legs at the same distance (about 16 mm) from the surface regardless of the platform's orientation, indicating that they can assess the distance to the platform without touching it. Since honeybees rely on visual cues to regulate flight speed during landing (Srinivasan *et al.*, 2000; Baird *et al.*, 2013), it is possible that they compute and control the leg extension distance using this sense as well but more detailed

experiments are required to investigate if this is indeed the case. It is also possible that bees rely on other sensory information to initiate leg extension, such as airflow (mechanosensory cues) created by the flapping wings and deflected back from the platform.

Bumblebees, just like honeybees, are efficient pollinating insects. To investigate how these insects orchestrate the final, critical moments of landing, bumblebees (*B. terrestris*) were trained to land on a similar platform as above, which could be tilted at different orientations (Reber *et al.*, 2016a: Paper 2) (Fig. 9).

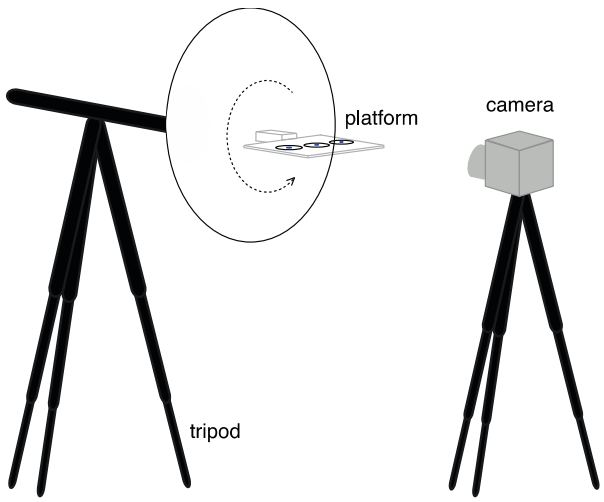


Fig. 9
Experimental setup where bumblebees were trained to feed from three circles of filter paper, drenched in sugar solution, and placed on a flat platform that was attached to a rotatable arm on a tripod. The landings were filmed from the side by a high-speed camera. Adapted from Reber et al (2016a)

Similar to honeybees, bumblebees also extended their legs at a constant distance (about 8 mm) from the surface, at least when the platform was tilted at 60 to 180° relative to the horizontal plane (Reber *et al.*, 2016a: Paper 2). At lower platform tilts (0° and 30°), the bumblebees extended their legs further away from the surface (Fig. 10). Considering that typical bumblebee flowers, such as dead nettle, foxglove and clover (Willmer, 2011) seldom have a perfectly horizontal surface, bumblebees may not be used to landing on this surface orientation. Hence, the earlier leg extension might be a safety mechanism to ensure that they extend the legs in time to avoid collision with the surface. However, more detailed experiments are necessary to explore this possibility.

The timing of leg extension during landing has also previously been investigated in the bumblebee *B. terrestris* and the nocturnal rainforest bee *M. genalis* with the aim to

study the effect of light intensity on landing precision in these bees (Baird *et al.*, 2015), the topic to which we will turn next.

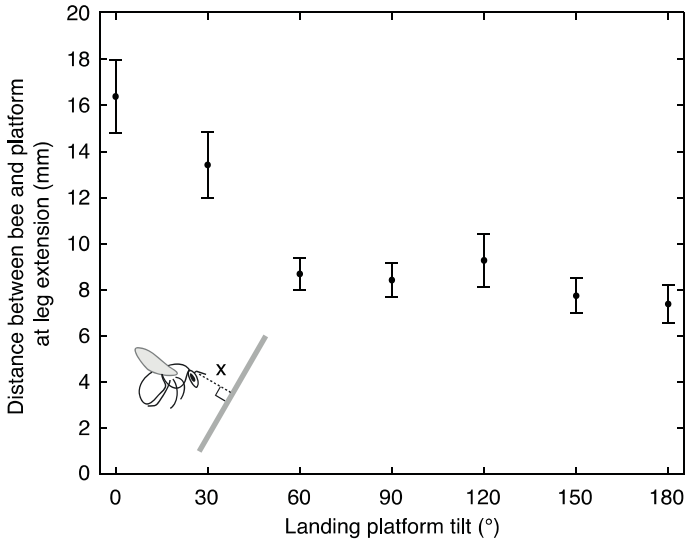


Fig. 10
The perpendicular distance (distance *x*, shown by the inset) between the base of the antennae and the platform surface at the moment of leg extension. Values are mean ± standard error of mean. For more details, see Reber et al (2016a)

Landing in dim light

As mentioned above (*see Flight control in dim light*), the landing trajectories of the nocturnal bee *M. genalis* at its nest become more meandering as light levels fall (Theobald *et al.*, 2007). Interestingly, the landing precision (measured as the time between leg extension and contact with the nest surface) did not change as light levels fell from 3.58 lx to 0.0018 lx, indicating that vision might not play a vital role during landing in these bees (Baird *et al.*, 2015). To further investigate this, disks carrying different visual patterns were attached to the nest entrances. One disk had a concentric black and white ring pattern, providing the bees with strong visual expansion cues as they approached the nest, whereas the other had a black and white radial (that is, divided into triangular sectors) pattern that minimised the optic flow generated by an approach. In this experiment, the bees extended their legs later when they approached the radial pattern, suggesting that it becomes more difficult for the bees to estimate the distance to the surface during approach to this pattern. Thus, *M. genalis* do appear to rely on visual expansion cues during landing but the timing of the leg extension is not affected by light intensity. This suggests that *M. genalis* still see

enough visual information to extend their legs in time or that they rely on additional sensory cues to initiate landing. Further experiments are needed to elucidate this.

To obtain a deeper understanding of insect landing behaviour in dim light, we characterized the landing performance of bumblebees, *B. terrestris,* while landing on a flat platform at two different orientations (0° and 90° relative to the horizontal plane) and at four different light intensities (ranging from 600 lx down to 19 lx) (Reber *et al.*, 2016b: Paper 3). As light levels fell, the bees oriented their bodies more vertically and their heads more horizontally with respect to the horizontal plane, suggesting that vision plays a role in body posture during landing in these bees. However, the changes in body posture were relatively small and the landings were still well controlled at the dimmer light levels.

Some insects, such as honeybees and bumblebees tend to hover, i.e. remain in the same place in the air for a short period of time before landing (Evangelista *et al.*, 2009; Reber *et al.*, 2016a: Paper 2; Reber *et al.*, 2016b: Paper 3). The purpose of this hover phase is probably to evaluate whether flowers are rewarding or not (Goulson *et al.*, 2001), a discrimination task that might be achieved by visual inspection of the pollen content of the flower (Zimmerman, 1982) or the specular reflections from nectar droplets (Kevan, 1976). If this task is mediated by visual cues, the ability of the bees to assess the distance to the surface during the hover phase could be affected in dim light due to the reduced reliability of visual information. Surprisingly, the bees hovered at the same distance from the platform across all light intensities (Reber *et al.*, 2016b: Paper 3), suggesting that their visual system is sensitive enough to reliably estimate the distance to the platform across the range of light levels tested. One way for the visual system to collect more light and improve the reliability of the visual signal before making the final touchdown at the surface would be to increase the duration of the hover phase. However, the hover duration remained constant regardless of light intensity (Reber *et al.*, 2016b: Paper 3). It is possible that the bumblebees increase their visual sensitivity in some other way, or perhaps rely on other cues, such as mechanosensory cues (as discussed above) to assess the distance to the platform.

As light intensity decreased, the bumblebees extended their legs further away from the platform, at least when it was oriented vertically (Fig. 11). If the bees rely on visual input to initiate leg extension, the earlier extension at lower light levels might be a behavioural adaptation to dim light. By extending the legs further away, the bees will reduce the risk of colliding with the surface as an effect of possible estimation errors in dim light. This type of error could be observed in the bees that flew along the tunnel midline at different light intensities in my earlier study on flight control in bumblebees (demonstrated by a loss in precision) (Reber *et al.*, 2015: Paper 1, *see Flight control in dim light*). Interestingly, the landing bees did not extend their legs earlier when the platform was horizontally oriented, suggesting that it is not necessary

to take precaution when approaching a surface from above. After all, at this orientation, the bees are in no danger of flying directly into it. Moreover, when the bees landed at the vertical surface, the time to contact (defined as the time between leg extension and first contact with the surface) increased as light intensity fell. This longer time to contact may be due to the greater distance to the platform at which leg extension was initiated but it may also depend on a slower approach speed. However, analysis of the flight speed revealed that the bees approached the platform at the same speed regardless of light intensity, indicating that the longer time to contact in dim light was simply an effect of the larger distance to the surface at which the legs were extended.

It is interesting that the bees did not slow down as light levels fell during the final moments of landing as they did when flying through the experimental tunnel (Reber *et al.*, 2015: Paper 1). This suggests that the bees do not adapt to dim light during landing by reducing speed, either because their visual system is sensitive enough without a behavioural adaptation or because they already fly at such low speed that a further reduction would not be of any advantage. In fact, during landing, the speed of the bees is much lower than when they fly through the tunnel (6 cm s^{-1} during landing vs. 89 cm s^{-1} during flight through the tunnel). Insects, such as flies (Kress and Egelhaaf, 2012), tend to move slower during walking than during flight. Thus, it would be interesting to know if walking insects adapt to dim light by walking more slowly, just like some flying insects do (Rose and Menzel, 1981; Spiewok and Schmolz, 2005; Reber *et al.*, 2015: Paper 1) or if they already move slowly enough to see sufficient visual information. To test this, we studied the orientation performance in dung beetles at different light intensities (Smolka *et al.*, 2016: Paper 4) and this is the topic of the next chapter.

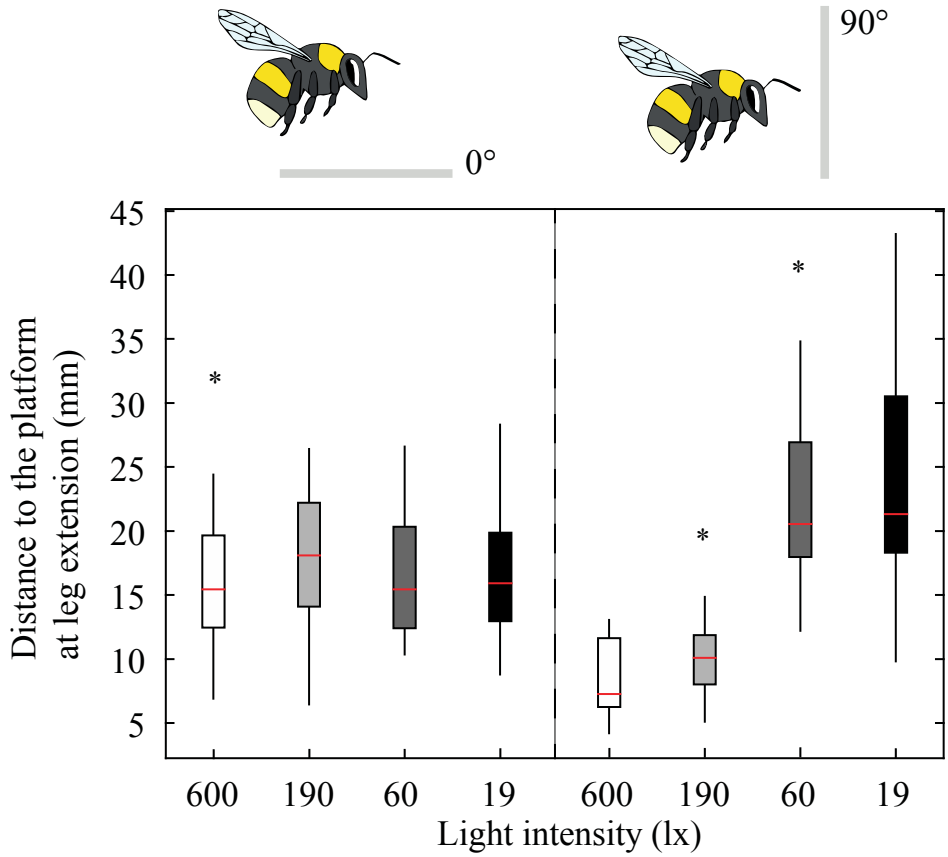
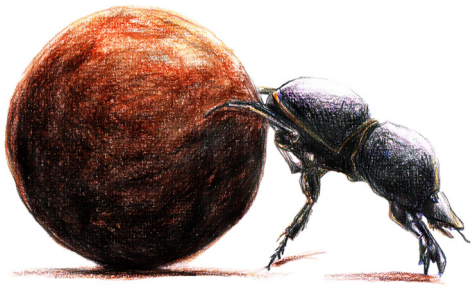


Fig. 11
Effect of light intensity and platform tilt on the distance to the platform at leg extension of bumblebees landing on a platform at two different orientations (0° and 90°) and at four different light intensities (600 lx, 190 lx, 60 lx, and 19 lx). The schematic drawings of the bees and the platforms depict the orientation of the platform. The edges of the boxes denote the 25th and the 75th percentiles, the central red mark is the median, the whiskers extend to the most extreme data points, and the black stars indicate outliers. For more details, see Reber et al (2016b)

Walking in dim light



Many walking, as well as flying insects rely on visual information to negotiate their path and to move in the right direction. As light intensity falls and visual information becomes less reliable, these tasks become more difficult to master (*see Vision in dim light*). Theoretically, the solutions to counteract these visual challenges should not differ depending on the form of transport (flying or walking) that carries the animal forward. As with flying insects (*see Flight control in dim light*), many ground-borne insects reduce their speed as light levels fall (Erber *et al.*, 2006; Kress and Egelhaaf, 2012; Narendra *et al.*, 2013; Otálora-Luna *et al.*, 2013), but there are interesting exceptions to this rule (Smolka *et al.*, 2016: Paper 4).

An example of an insect that walks in the dark is the nocturnal Australian bullant *M. pyriformis*, where most of the workers leave the nest during the evening hours on a quest to forage on *Eucalyptus* trees (Narendra *et al.*, 2010). Here, the ants catch prey and feed on honeydew produced by insects living in the trees and, when morning comes, they return to the nest with the prey. Similar to bumblebees that fly slower and along more winding flight paths as light intensity decreases (Reber *et al.*, 2015: Paper 1), the ants walk more slowly and their walking paths become more tortuous in the dark (Narendra *et al.*, 2013). In addition, the ants also pause more often and for a longer time. Together, these results suggest that the visual homing efficiency of the ants is compromised in dim light and this might be the reason to why most of the workers leave and return to the nest during twilight and not in the middle of the night. Since temperature is a factor that usually affects walking speed in insects (Heatwole, 1996), one could argue that the ants walk more slowly simply because of the lower temperature at night. The walking speed of the ants is however normally constant at temperatures from 5 to 30°C (Jayatilaka *et al.*, 2011), a range that the

temperature kept well within during the nights of the observations (Narendra *et al.*, 2013). The longer pauses might be a behavioural adaptation to dim light, allowing the ants to gather more light and increase their visual sensitivity, similar to nocturnal spiders (Nørgaard *et al.*, 2008).

The effect of light intensity on walking has also been addressed in the lab, directly or indirectly, in a number of different insects (Erber *et al.*, 2006; Kress and Egelhaaf, 2012; Otálora-Luna *et al.*, 2013). The phototactic behaviour of honeybees was characterized by measuring walking times, path lengths, and walking speed when moving towards LEDs of different light intensity in an otherwise dark arena (Erber *et al.*, 2006). As the light intensity of the light stimulus decreased, the walking paths of the bees became more winding and they walked more slowly. In an experiment that was designed to investigate head and body stabilization in blowflies (*C. vicinia*) during walks on differently structured substrates, it was also found that the walking style of the flies changes when they walk in full darkness (Kress and Egelhaaf, 2012). In the dark, the flies pitched their body and head upward. The shift of the body weight towards the middle and hind legs made it possible for the front legs to be used as tactile probes. The flies also walked significantly slower in the dark compared to in bright light. When male tropical root weevils, *Diaprepes abbreviatus,* walk in complete darkness, they also decrease their speed and their paths become more tortuous compared to when they are stimulated with light emitted by LEDs (Otálora-Luna *et al.*, 2013). Together, these studies indicate that walking, as well as flying insects employ a temporal summation strategy for improved sensitivity in the dark and compensate for the loss in temporal resolution by reducing the speed of movement.

Nocturnal, ball-rolling dung beetles fly, walk, and roll their balls in the dark. When they have located a pile of dung, they carve out a piece of it with their fore legs and construct a ball, which they quickly roll away backwards with their heads down and their hind legs on the ball. At some distance away from the dung pile, they bury the ball underground to eat it in peace (Hanski and Cambefort, 1991). While the flight performance of these insects has never been studied in detail, they are well known for their ability to orient while walking at night.

It turns out that the paths of the ball-rolling dung beetles are remarkably straight, presumably to effectively escape from the competition at the dung pile (Byrne *et al.*, 2003). When rolling their ball backwards, the beetles cannot see or try to avoid obstacles, but it is of utter most importance that they stay on their course away from the dung pat. For this, both nocturnal and diurnal dung beetles rely on celestial cues, such as the position of the sun or the moon (Byrne *et al.*, 2003; Dacke *et al.*, 2004; Dacke *et al.*, 2014), the sun's or the moon's polarization pattern (Byrne *et al.*, 2003; Dacke *et al.*, 2003a; Dacke *et al.*, 2003b; el Jundi *et al.*, 2014; el Jundi *et al.*, 2015), the intensity gradient of the sky (el Jundi *et al.*, 2014), or the Milky way (Dacke *et al.*, 2013). They detect these celestial cues through one (or sometimes two) pairs of

superposition compound eyes (McIntyre and Caveney, 1998). As described earlier (*see Insect eyes*), this type of eye has a superposition aperture and is therefore much more sensitive to light than an apposition eye of the same size. In addition, nocturnal dung beetles have optical adaptations, such as larger facets and wider rhabdoms, to further increase their sensitivity to light (Caveney and McIntyre, 1981; Warrant and McIntyre, 1990; Warrant and McIntyre, 1991; Dacke *et al.*, 2003b; McIntyre and Caveney, 1998; Frederiksen and Warrant, 2008; Byrne and Dacke, 2011). Still, these improvements for increased sensitivity is not sufficient to fully meet the changes in light level between day and night. Therefore, it is possible that nocturnal dung beetles also engage in further retinal adaptations, such as increasing the integration time of the photoreceptors (like bumblebees, Reber *et al.*, 2015: Paper 1) or the photoreceptor gain (Frederiksen, 2008) or higher neural summation strategies, such as temporal and spatial summation of the visual signals.

To investigate the possible effect of falling light levels on orientation (and walking) in ball-rolling dung beetles, we compared the performance of diurnal (*Scarabaeus lamarcki*) and nocturnal (*Scarabaeus satyrus*) dung beetles (Smolka *et al.*, 2016: Paper 4). The beetles were filmed as they exited a circular arena, both outdoors in their natural habitat and under controlled conditions in the lab. We found that both species oriented well when they had a point light source to rely on, the moon outdoors or a lamp indoors. However, when the moon was not visible, the nocturnal beetles were much better oriented than their diurnal counterparts (whose paths became significantly less straight) (Fig. 12). To roll straight under these conditions, the beetles have to rely on the polarization pattern around the moon or the constellation of stars (Dacke *et al.*, 2003a; Dacke *et al.*, 2013). Our results suggest that the nocturnal beetles are better equipped for this task due to their larger eyes and more sensitive visual systems, as well as the larger dorsal rim area (the area in the eye that is specialized to detect polarized light) compared to diurnal beetles (Dacke *et al.*, 2003b). However, when the beetles were tested in the lab (where only a lamp and no wide-field cues were available), the paths of both species became more tortuous as light intensity decreased, highlighting the role of wide-field cues for straight-line orientation in the nocturnal beetles.

Interestingly, we found no evidence that ball-rolling speed is related to light intensity in either species, indoors or outdoors (Fig. 13). The diurnal beetles rolled significantly slower than the nocturnal beetles in the lab and at night in the field but this is probably due to lower temperatures, which diurnal beetles might not be as physiologically adapted to as nocturnal beetles. The lack of effect of light intensity suggests that dung beetles do not suffer from temporal blur at these speeds. It is possible, however, that they engage in temporal summation to some extent but only with integration times that are short enough not to interfere with the sky compass system. If the beetles were moving faster though, such as during flight, their visual system might be more affected by the dim light, and it might be the case that dung

beetles fly slower in dim light, as bumblebees do (Reber *et al.*, 2015: Paper 1). This would be interesting to investigate in the future.

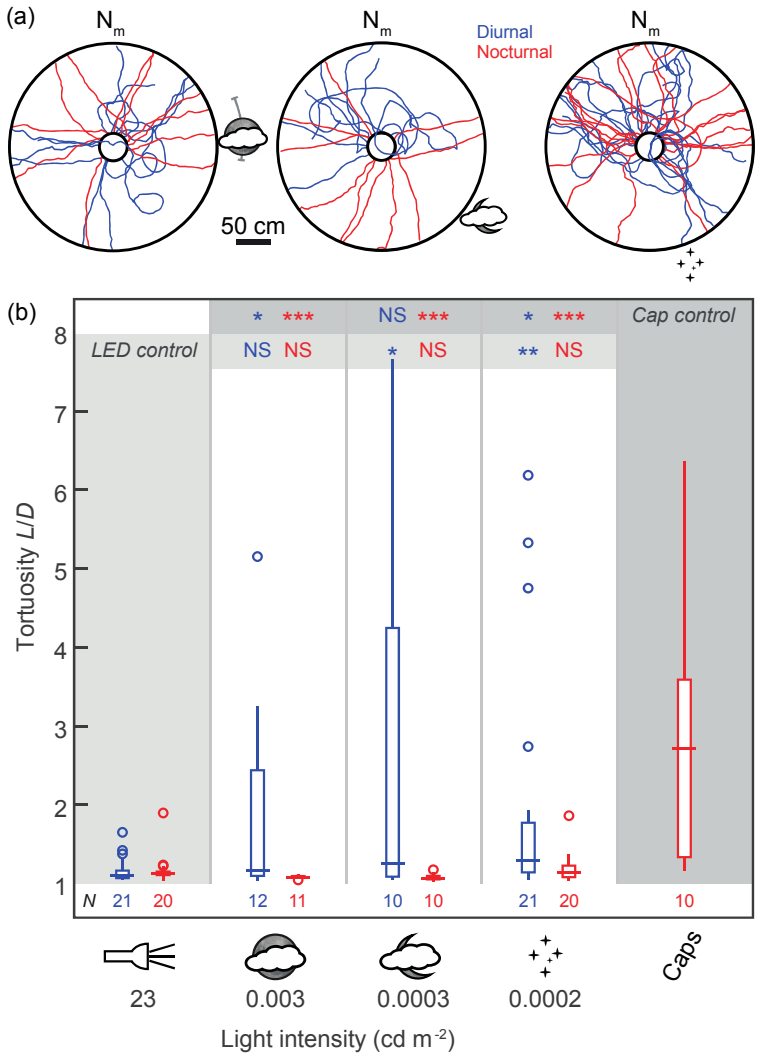


Fig. 12
Diurnal beetles roll less straight when only the polarized light around the moon or the stars are visible, whereas nocturnal beetles still roll straight in these conditions. (a) The paths of diurnal (blue) and nocturnal (red) dung beetles while exiting a circular arena (seen from above) in three different conditions: on a full moon night with the moon shaded, on a crescent moon night with the moon shaded, and on a night with only starlight. N_m = local magnetic north. (b) Path tortuosity. Nocturnal beetles (red) rolled equally straight in all conditions, whereas diurnal beetles (blue) rolled less straight when the crescent moon was shaded and when only starlight was visible compared to the LED control (left column, see lightly shaded line for statistics). When compared to the cap control (nocturnal beetles with a cap that blocks the view of the sky, right column, see darker shaded line for statistics), both nocturnal and diurnal beetles roll straighter in most of the conditions when wide field cues are available, suggesting that diurnal beetles are still well oriented when they have a view of the sky. Boxplots show median, interquartile range and outliers (any points more than 1.5 interquartile ranges above the 75th percentile or below the 25th percentile). For more details, see Smolka *et al.* (2016)

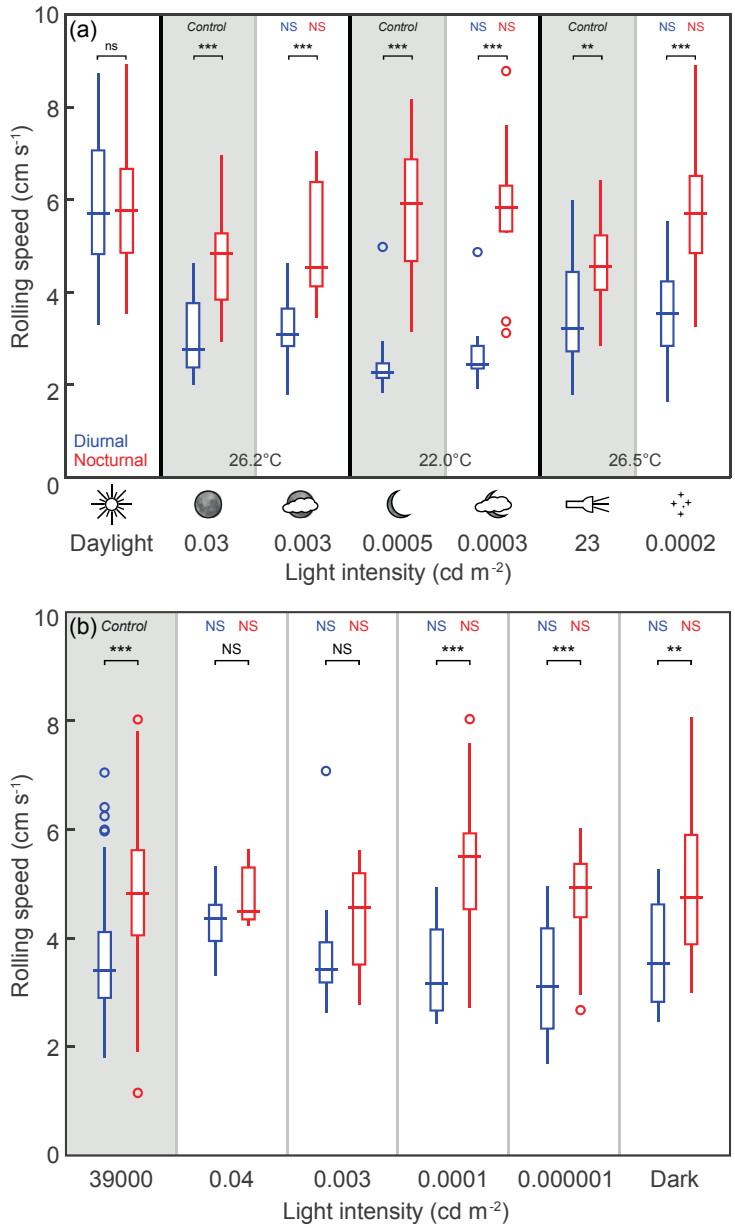


Fig. 13
The ball-rolling speed of dung beetles is not affected by light intensity. (a) Rolling speeds in the field. During the day, the speed was the same for diurnal (blue) and nocturnal (red) beetles. At night, in all conditions, nocturnal beetles rolled significantly faster than diurnal beetles (black significance markers), but neither species rolled more slowly in the dark (compared to suitable controls, blue/red significance markers). (b) Rolling speeds in the lab. Nocturnal beetles rolled significantly faster than diurnal beetles in most conditions, but there was no effect of light intensity on rolling speed in neither species. Boxplots show median, interquartile range and outliers (any points more than 1.5 interquartile ranges above the 75th percentile or below the 25th percentile). For more details, see Smolka *et al.* (2016)

Another strategy for increasing sensitivity to light at night is spatial summation, in which single neurons pool signals from many photoreceptors (*see Optical and neural adaptations*). A recent study shows that nocturnal dung beetles rely primarily on the polarized light around the moon, rather than the moon itself to orient along straight paths at night (el Jundi *et al.*, 2015). This might be because this wide-field cue supports summation of spatial information from a larger visual field compared to the point light source that the moon offers.

In summary, we can conclude that walking and flying insects seem to employ similar strategies for improved sensitivity in the dark, but since walking in general is a slower mode of transport than flight, ground-borne insects with superposition eyes do not necessarily need to compensate for a potential loss in temporal resolution by a reduction in speed.

Conclusions

To move in dim light, many insects have evolved visual adaptations to increase their visual sensitivity as light levels fall. Bumblebees for example integrate the visual signal for a longer period of time in their photoreceptors as light levels fall (Paper 1). To compensate for the increased visual blur that this fast-moving insect experiences as a consequence of this temporal summation, bumblebees gradually reduce their flight speed as light levels fall (Paper 1). This behavioural adaptation supporting movement in the dark has also been observed in honeybees, hornets and blowflies, as well as in walking ants and root weevils.

In contrast, the nocturnal tropical bee *M. genalis* (flying) and dung beetles (walking) (Paper 4) do not slow down as light levels fall, suggesting that they do not employ temporal summation to increase light sensitivity (or only with integration times that are short enough not to interfere with the visual information that they extract). It is likely that these insects instead preferentially engage spatial summation to increase their sensitivity to light.

As light levels fall, the flight or walking paths of most insects become more meandering (Paper 1). This indicates that the visual adaptations these insects employ do not support perfect course control in dim light. Nocturnal beetles, on the other hand, still roll their balls along straight tracks even on the darkest of nights (paper 4). This is most likely because the cues used to guide these insects over the savannah (the polarization pattern formed around the moon or the Milky Way) extend over the entire sky. These wide-field cues thus seem to allow the beetle to integrate visual signals over a wide visual field, thus improving sensitivity without a (measurable) loss in orientation precision.

To safely end their forward movements in dim light, a walking insect can simply stop in its stride, while a flying insect needs to descend towards a surface and land. Bumblebees are able to perform well-controlled landings under a wide range of light intensities (Paper 2 and 3) and orientations of the landing surface (Paper 2). The finding that body posture and the moment of leg extension are affected by both platform tilt and light intensity indicates that these preparations for landing are mediated by visual cues. This sets the scene for more detailed experiments aimed to investigate the visual mechanisms for landing control in the dark. This can for example be done by training the bumblebees to land on disks (see Paper 2 and 3 for

method) with patterns providing the bees with high vs. low contrast cues, strong vs. weak visual expansion, or small field vs. large field cues for landing.

In summary, the research presented in this thesis has increased our knowledge of how locomotion in flying, landing, as well as walking insects is affected by light intensity. These findings have also raised interesting questions for the future. The deeper understanding we have gained from this research concerning the performance of small insect nervous systems is not only interesting from a biological point of view, but is also interesting for technical reasons. The data presented here has the potential to help engineers to derive algorithms useful in the development of small robots, which are constantly being improved for better efficiency across a range of light intensities in natural and artificial environments.

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Att färdas i mörker: insekters lösningar, vinster och kostnader

In sin ständiga jakt på mat måste många insekter undvika att krocka med grenar, stenar och blad, samt färdas i en lämplig kompassriktning. Åtskilliga insekter använder sin syn för att kontrollera detta. En nektarsökande humla, till exempel, reglerar sin flyghastighet efter hur snabbt föremål i omgivningen rör sig över dess ögon. Denna typ av flygkontroll, och alla andra synstyrda beteenden, kräver dessvärre en viss mängd ljus för att fungera optimalt och när det blir mörkare blir det svårare att uppnå denna ljusmängd. För att maximera sitt ljusintag har många skymningsaktiva och nattaktiva insekter därför större ögon och större fasetter än sina dagaktiva släktingar. Några har dessutom utvecklat förmågan att neuronalt lägga ihop ljusintag från flera områden av synfältet (spatial integrering) eller att bearbeta ljussignalen i varje syncell (fotoreceptor) under en längre tid (temporal integrering). Men allt har ett pris och dessa strategier leder ofta till en mindre detaljrik bild av verkligheten eller att föremål som rör sig snabbt inte längre går att se. Syftet med den här avhandlingen är att beskriva de anpassningar som insekter har utvecklat för att öka sin ljuskänslighet och att öka vår förståelse för hur insekters flygkontroll, landningsprecision och orienteringsförmåga påverkas av fallande ljusnivåer.

I dagsljus flyger en humla i mina försök elegant och säkert genom en 30 cm bred tunnel klädd med schackmönster. En serie välkontrollerade studier visar att de fortsätter att göra så även i mycket svagt ljus, men med lägre flyghastighet efterhand som det blir mörkare. Samtidigt kan vi visa att en fallande ljusintensitet även påverkar fotoreceptorernas reaktionsförmåga, som även den gradvis blir långsammare efterhand som mörkret faller. Humlor anpassar på så vis sin syn genom att fånga upp ljus under en längre tidsenhet med ökad ljuskänslighet som vinst. Kostnaden humlorna betalar är ett långsammare synsystem. Om de fortsätter att flyga med samma hastighet riskerar de därför att komma farligt nära en av tunnelns väggar utan att upptäcka den i tid. Ett sätt att kompensera för detta är helt enkelt att flyga långsammare när det blir mörkare. Detta är också precis vad jag observerar.

För att bättre förstå insekters landningsbeteende tränade jag även humlor att landa på en plattform som gick att rotera. Det visar sig att humlor justerar sin kroppshållning och sina antenner efter plattformens vinkel, samt sträcker ut benen på ett konstant

avstånd från plattformen. Dessa resultat tyder på att humlorna bedömer var plattformen är och hur den är orienterad utan att vidröra den, förmodligen genom att förlita sig på sin syn, men antennerna kan också ha en roll med i spelet. Även under mycket mörka förhållanden, på gränsen till vad humlorna kan flyga under, är deras landningar välkontrollerade. Jag observerade inte en enda humla som krockade med plattformen under mina försök. När det blir mörkt justerar dock humlorna kroppen mer vertikalt och huvudet mer horisontellt i förhållande till horisontalplanet, samt sträcker ut benen tidigare jämfört med mer ljusa förhållanden. Förmodligen är detta anpassningar som gör det möjligt för dem att landa mjukt även under ljussvaga förhållanden.

I min avhandling utforskar jag även effekten av ljusintensitet på orienteringsförmåga och transporthastighet hos gående insekter. Detta gör jag genom att låta dagaktiva och nattaktiva dyngbaggar rulla sina dyngbollar ut från mitten av en rund arena i Sydafrika, samt under mer kontrollerade former i labbet. Det visar sig att dagaktiva och nattaktiva dyngbaggar orienterar sig med samma precision så länge de erbjuds en punktljuskälla, såsom månen, solen eller en lampa som referenspunkt. När däremot endast stjärnhimlen eller månens polarisationsmönster går att se överträffar de nattaktiva dyngbaggarna sina dagaktiva släktingar. Dessutom fortsätter båda arterna, oberoende av ljusintensitet, att rulla sina bollar över savannen med samma imponerande hastighet. Detta tyder på att dyngbaggarna inte bearbetar synsignaler långsammare, utan snarare lägger ihop synsignaler från större delar av synfältet för att öka sin ljuskänslighet.

Sammanfattningsvis bidrar min avhandling till ökade kunskaper om hur synstyrda beteenden hos flygande, landande och gående insekter påverkas av rådande ljusförhållanden och vilka anpassningar de utvecklat för att utföra dem även när det blir mörkare.

Tack

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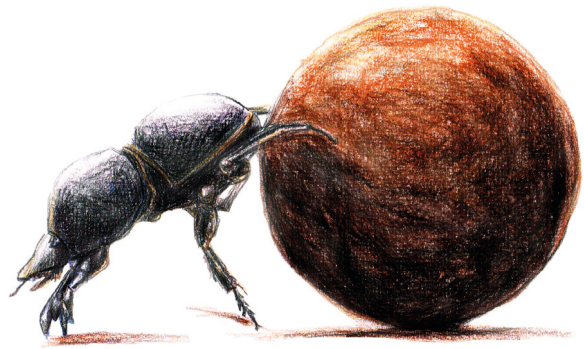

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