

Twilight orientation to polarised light in the crepuscular dung beetle *Scarabaeus zambesianus*

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Summary

The polarisation pattern of skylight offers many arthropods a reference for visual compass orientation. The dung beetle *Scarabaeus zambesianus* starts foraging at around sunset. After locating a source of fresh droppings, it forms a ball of dung and rolls it off at high speed to escape competition at and around the dung pile. Using behavioural experiments in the field and in the laboratory, we show that the beetle is able to roll along a straight path by using the polarised light pattern of evening skylight. The receptors used to detect this skylight cue can be found in the ommatidia of the dorsal rim area of the eye, whose structures differ from the regular ommatidia in the rest of the eye. The dorsal rim ommatidia are characterised by rhabdoms with microvilli oriented at only two orthogonal

orientations. Together with the finding that the receptors do not twist along the length of the rhabdom, this indicates that the photoreceptors of the dorsal rim area are polarisation sensitive. Large rhabdoms, a reflecting tracheal sheath and a lack of screening pigments make this area of the eye well adapted for polarised light detection at low light levels. The fan-shaped arrangement of receptors over the dorsal rim area was previously believed to be an adaptation to polarised light analysis, but here we argue that it is simply a consequence of the way that the eye is built.

Key words: dung beetle, *Scarabaeus zambesianus*, orientation, ommatidia, receptor, rhabdom, polarisation pattern, skylight.

Introduction

During twilight, the sky completely changes its appearance. The intensity of light drops by 7–9 log units and the red glow from the setting sun is slowly replaced by the pale star shine from other more distant stars. This marked decline in illuminance accelerates when the sun is still visible above the horizon and marks the onset of twilight. It lasts until the sun has disappeared some 20° below the horizon (Rozenberg, 1966). On some evenings, this period of transition is accompanied by the rise of the moon.

Also present in the sky, but not visible to humans, is the pattern of polarised skylight centred on the sun. During the course of the day, this pattern of polarised light changes its appearance with the apparent movement of the sun. During twilight, the pattern is most simple, with the light of the whole sky polarised in one direction. The zenith of the sky now has the highest degree of polarisation of the day, reaching between 70% and 80% polarisation (Brines and Gould, 1982; Wehner, 1989). This high degree of polarization stretches in a band across the sky from south to north. The remainder of the skylight is polarised in a parallel direction with falling degrees of polarisation towards the sun and the anti-sun. On nights with a full moon, a similar pattern of polarised light will also form around this source of light (Gál et al., 2001).

In the compound eyes of beetles and other arthropods, the

microvilli of the retinula cells form light-absorbing rhabdoms. In polarisation-sensitive insects, the arrangement of these structures follows a common pattern; the microvilli in each rhabdomere are organised in only one of two orthogonal directions (for a review, see Labhart and Meyer, 1999). With a maximum sensitivity to light polarised parallel to the direction of the microvilli (Goldsmith and Wehner, 1977; Hardie, 1984; Israelachvili and Wilson, 1976), this arrangement tunes the two groups of receptors to orthogonal planes of polarisation. An opponency between the two sets of receptors will not only enhance the polarisation contrast but will also make the system independent of the light intensity of the stimulus (Labhart, 1988; Nilsson and Warrant, 1999). This rhabdom design is generally confined to a narrow strip at the dorsal rim of the eye, termed the dorsal rim area (DRA; Labhart, 1980). Within this area, there are often additional specialisations to facilitate the perception of the polarised light in the sky. Examples of such are a lack of screening pigments or poor lens optics (Aepli et al., 1985; Burghause, 1979; Labhart et al., 1992; Meyer and Labhart, 1981, 1993; Ukhanov et al., 1996).

As the day slips towards night, many animals begin – or end – their activity. The dung beetle *Scarabaeus zambesianus* starts to fly at around sunset with the prospect of finding fresh dung.

Once found, it forms a ball of dung and rolls it off at high speed in a line as straight as the terrain will allow. This is supposedly done to avoid competition at and around the food source. The ball is finally buried in a suitable place to be consumed in secure solitude, either by the beetle itself or by a beetle larva (Hanski and Cambefort, 1991). While rolling, the beetle inevitably has to rely upon some sort of reference to stay on route. For many animals, this cue is the polarisation pattern of skylight, a stimulus well known to be used for orientation (see Waterman, 1981).

Whereas diurnal polarised light orientation has been thoroughly explored, twilight orientation remains a rarely investigated topic. An exception is the use of skylight polarization in dusk-migrating birds (Helbig, 1990, 1991; Moore and Philips, 1988; Philips and Moore, 1992), cockchafers (Labhart et al., 1992) and spiders (Dacke et al., 1999, 2001). The mechanism behind the perception of polarised light in birds, however, remains unsolved, and some studies even argue against a use of skylight polarisation as a cue (Coemans et al., 1994). Dusk-active bumblebees have also been suggested to navigate using the polarised light pattern at dusk, when the surroundings are too dim to distinguish terrestrial landmarks (Wellington, 1974). The morphological basis of this behaviour has, to our knowledge, not yet been investigated.

In the present study, we combine the results of behavioural studies of polarised light orientation in the field with those of morphological studies of the eyes of the crepuscular dung beetle *Scarabaeus zambesianus*. By restricting our experiments to moonless evenings, the beetles could only use direct sunlight and the polarized light pattern of skylight for celestial orientation. We show that polarised light in the sky is used by beetles for orientation to roll balls in straight paths, and we identify the receptors used to perceive this skylight cue. Based on these results, we also question whether the fan-shaped arrangement of analysers in the DRA is an adaptation to polarised light orientation.

Materials and methods

Experimental sites and animals

Adult male and female *Scarabaeus zambesianus* Péringuey (Coleoptera: Scarabaeidae) were collected at Cinergy Game Farm, Naboomspruit District, Mpumalanga Province, South Africa, 24°38' S, 28°45' E. Field experiments were conducted in the same area on evenings when the beetles were spontaneously active. Flying beetles were attracted to the experimental site by the use of 1 litre of fresh pig dung, placed in the centre of an arena 3 m in diameter. The period of activity was determined from the time period during which the beetles arrived at the dung pile. The sandy ground in the arena was carefully levelled and any objects that could function as landmarks were removed. All manipulations on the beetles were performed within this circle, as soon as the beetle had formed a ball and started to roll it away from the pile of dung. To ensure that no beetles were ever used twice in the same

experiment, the beetles were individually marked with a number on the thorax using a Liquid-paper™ pen. The number of beetles in each experiment is thus equivalent to the number of runs. Laboratory experiments were conducted in Lund, Sweden within two weeks of capturing the beetles. Adult male and female *Pachysoma striatum* Castelnau (Coleoptera: Scarabaeidae) were collected in Kleinsee, Namaqualand, South Africa, 29°33' S, 17°10' E.

Polarised light navigation

In a simple set of experiments, the beetles were allowed to roll along their chosen course with an undisturbed view of the evening sky. A semitransparent Perspex barrier (7 cm high × 30 cm wide) was placed perpendicular to the path of the beetle, forcing the beetle to move around it. The deviation after the obstacle, from the path taken before the obstacle, was measured with a protractor using the tracks made by the beetles in the fine sand as indicators of the path. All measurements were taken within the first hour after sunset on moonless nights.

In a second experiment, a polarising filter (Polaroid HN 22) with a circular diameter of 42 cm was mounted in a holder

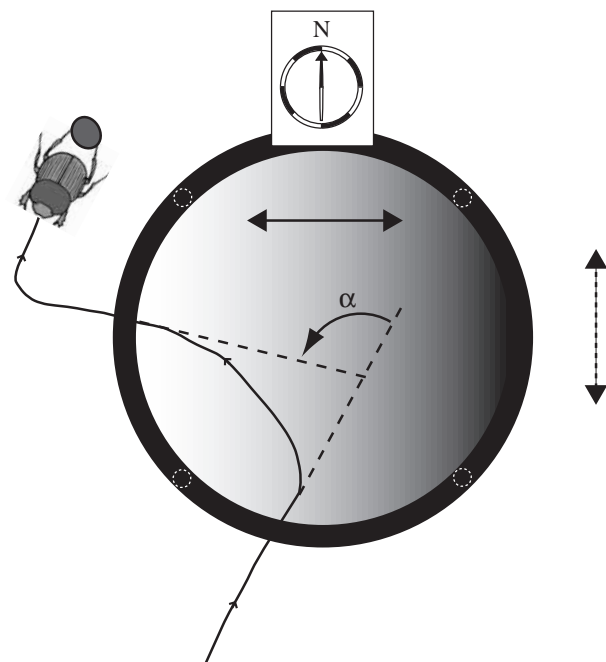


Fig. 1. Set-up used for experiments on the orientation to polarised light. The black open circle represents the polarising filter (shaded) in its holder, and the white dotted circles represent four symmetrically placed legs, height 10 cm. The set-up was equipped with a magnetic compass to orient the e-vector produced by the filter (double-headed arrow) in a west-easterly direction, 90° to the e-vector on the evening skylight (dotted double-headed arrow). The turn made by the beetle (α) in response to the shifted polarisation pattern experienced on entering the filter was measured from the track drawn from filming the beetle. Open arrows mark the direction of movement. Note that the beetle rolls head down and backwards.

with four symmetrically placed legs, each with a height of 10 cm. With the help of a magnetic compass fitted on the holder, the filter was placed under the open sky over the expected path of the beetle with its e-vector transmission axis oriented in a west-easterly direction (Fig. 1). Thus, as the beetle entered the area below the filter, the south-northerly oriented polarised light pattern of evening skylight appeared to switch by 90°. The exact orientation of the filter had to be adjusted according to the azimuth of the setting sun. In our experiments, the sun set at 250° east of north and the transmission axis of the filter was oriented 70–250° east of north to simulate a 90° switch of the polarised twilight pattern of skylight. The reaction of the beetles to this switch was filmed using a Sony video camera equipped with 'night-shot' and was later analysed in the laboratory for tracing the path of the beetle. Both the empty holder and the holder with the filter with its e-vector transmission axis oriented in a south-northerly direction were placed over the expected path of the beetle to act as two controls. The reaction of the beetles to these controls was recorded and analysed as described above.

In the laboratory, we used the same filter and holder as described above, but the filter was now rotated within the holder. An Osram Ultra-Vitalux lamp (300 W) was used as a light source, centred above the filter. Tracing paper was placed on top of the polarising filter to present an extended polarised stimulus. A beetle and its ball were placed in an arena (70 cm×100 cm) covered with fine sand. As soon as the beetle had rolled 5 cm in under the filter, the filter was rotated through 90°. The angle turned by the beetle, in response to the filter being turned, was measured by analysing the track made by the beetle in the fine sand. As a control, the track made by the beetles under a stationary filter was also recorded. The direction taken towards the centre of the filter was compared with the direction taken after this point.

Histology

Sections for light and electron microscopy were prepared using conventional techniques. The eyes were dissected and fixed for 72 h at 8° in a fixative containing 2.5% glutaraldehyde, 2% paraformaldehyde and 0.1 mmol l⁻¹ EGTA in 0.1 mol l⁻¹ cacodylate buffer. The buffer was adjusted to pH 7.2. Following postfixation with 1% OsO₄ in 0.1 mol l⁻¹ cacodylate buffer for 2.5 h at room temperature, the tissue was dehydrated in an ethanol and propylene oxide series and embedded in Epon resin. A possible twist of the DRA rhabdoms was analysed by 0.5 µm cross-sections through the depth of the DRA. In these sections, a single rhabdom was identified during sectioning with the help of irregularities in the retina. The orientation of the transverse-axis of this rhabdom and neighbouring rhabdoms was determined every five sections. Ultra-thin sections (50 nm) were taken and prepared for electron microscopy with 1% uranyl acetate and lead citrate. Animals used for scanning electron microscopy were air-dried and sputter coated with gold-palladium (40/60).

Results

Time of activity

The sun at Naboomspruit set at 250° east of north between 18.58 h and 19.00 h at the time of our experiments. On evenings warm enough for the beetles to fly, they became active some time between 10 min before and 10 min after sunset. When no moon was available, they were active for 40–50 min, while on evenings with a high moon they stayed active for another 40–50 min. On completely overcast nights, no activity could be measured.

General behaviour

S. zambesianus starts forming a ball of dung immediately after landing on a pile of fresh dung. It does so by the use of its flat front legs. As soon as the ball is ready, the beetle pushes it off the pile and climbs on top of it. Here, it cleans its eyes, stretches its head out and performs one or two rotatory (yaw) body movements before it climbs down and starts rolling, head down, pushing the ball with its hind legs. If the beetle encounters a branch or a deep hole that makes it fall over, it often repeats its little dance on top of the ball before it starts rolling again. The ball is finally buried at a suitable spot some 5–30 m away. All balls rolled in this study were feeding balls to be consumed by the beetle itself. Once the ball of dung is depleted, the beetle will leave the burial spot never to return again.

Orientation to polarised light

With a full view of the moonless twilight sky, the beetles ($N=15$) negotiate an obstacle placed in their way by continuously pushing the ball sideways. As they reach the end of the obstacle they again set out parallel to the course they started with an absolute mean angular deviation from the

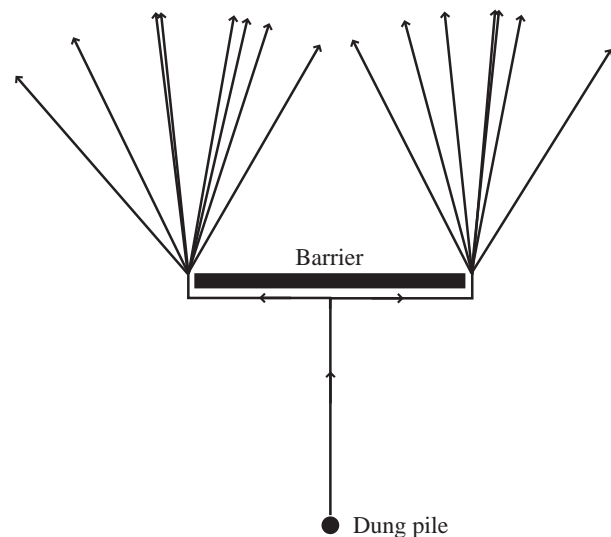


Fig. 2. Schematic drawing of the orientation of the beetles on encountering a barrier ($N=15$). Open arrows mark the direction of movement. Beyond the barrier, seven of the beetles turned to the left of the previous course, and eight turned to the right. Absolute mean angle of deviation from the original course is 16.9°.

original running direction of $16.9 \pm 11.5^\circ$ (mean \pm s.d.). Of 15 beetles, seven turned to the left from the original running direction and eight turned to the right (Fig. 2). Taking the directions of the deviations into consideration, where a left turn is assigned a 'minus' and a right turn is assigned a 'plus', there is no bias to the left or to the right ($-0.2 \pm 20.3^\circ$). This experiment clearly shows that, in general, beetles maintain their chosen course, deviating from it only temporarily when forced to do so.

In the second experiment, the natural polarisation pattern was manipulated *via* rotation by 90° as beetles ($N=26$) rolled their ball in under a polarising filter. The beetles continued to roll along their chosen course until they were at least 5 cm in under the filter. Without any delay, they then turned in response to the rotated polarisation pattern (Fig. 1). A mean turn of $80.9 \pm 15.8^\circ$ was close to the expected 90° . A small turn of $6.7 \pm 5.0^\circ$, recorded in response to rolling in under a polarising filter placed with its e-vector transmission axis oriented parallel to the e-vector of evening skylight, was not significantly different (*t*-test, $P > 0.05$) from the response when rolling in under the empty holder of the filter ($4.6 \pm 4.5^\circ$).

In the laboratory, where the beetles ($N=8$) were exposed to a polarising filter rotated through 90° , the turn was smaller, with a mean value of $61.4 \pm 16.1^\circ$. With a non-rotated filter, no turn was recorded.

Morphology of the eye and standard ommatidia

The eyes of *S. zambesianus* are divided into dorsal and ventral eyes by a cuticular ridge, the canthus, which projects from the edge of the 'cheek' (Fig. 3). The dorsal eye is the smaller of the two. Each of these eyes acts independently as a superposition eye. The surface of the eye is perfectly smooth without any visible facets. The thick corneal lens of each ommatidium is attached to a crystalline cone, beneath which is a clear zone and a $120 \mu\text{m}$ -long rhabdom. No screening pigments, but a tracheal tapetum, can be found between the rhabdoms as far as half way up the rhabdom. For the structures mentioned above, no differences were observed among different regions from the dorsal and ventral eyes. Microvilli from seven of eight retinula cells form the rhabdom in both eyes, but the arrangement of these structures varies across the eye. In the ventral eye and the ventral half of the dorsal eye, the microvilli form a flower-shaped rhabdom (Fig. 4B,C). Here, the microvilli run in different directions in different rhabdomeres.

Specialised ommatidia of the dorsal rim area

In the dorsal half of the dorsal eyes, the microvilli of the seven cells run in only two directions, forming an almost heart-shaped structure in cross-section (Figs 4A, 5). In these specialised ommatidia, the microvilli of receptor cell 1 run parallel to those of cell 1 in neighbouring ommatidia but perpendicular to the microvilli of the remaining cells (2–7) of the ommatidium (for cell numbering, see Labhart et al., 1992). This special eye region, termed the dorsal rim area (DRA; Labhart 1980), extends for approximately 26 ommatidia along

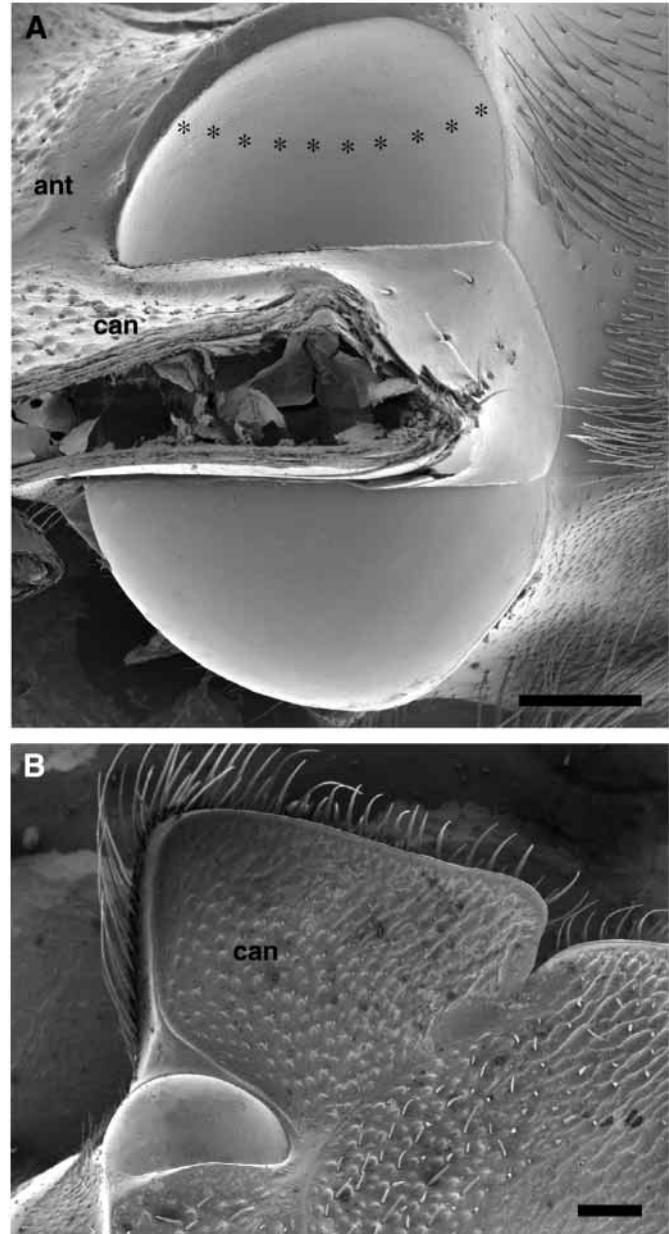


Fig. 3. (A) Scanning micrograph of the dorsal and ventral eye of *S. zambesianus*. For correct orientation, the anterior (ant) direction of the animal is indicated. A lateral view of the head of the beetle shows the canthus (can) that totally separates the eye into a dorsal and a ventral part. The asterisks mark the border of the dorsal rim area that covers approximately half the dorsal eye, narrowing towards the ends. (B) Scanning micrograph of the dorsal eye and canthus. Scale bars: $500 \mu\text{m}$.

the dorsal rim of the eye. With a width of approximately 20 ommatidia in the centre, narrowing towards the ends, the DRA covers roughly 50% of the dorsal eye (Fig. 3A). Serial cross-sectioning through the entire length of the rhabdoms indicates that there is no rhabdomeric twist or big jumps in microvillar orientation. Microvillar misalignment strongly influences the polarisation sensitivity as it makes the retinula cell less sensitive

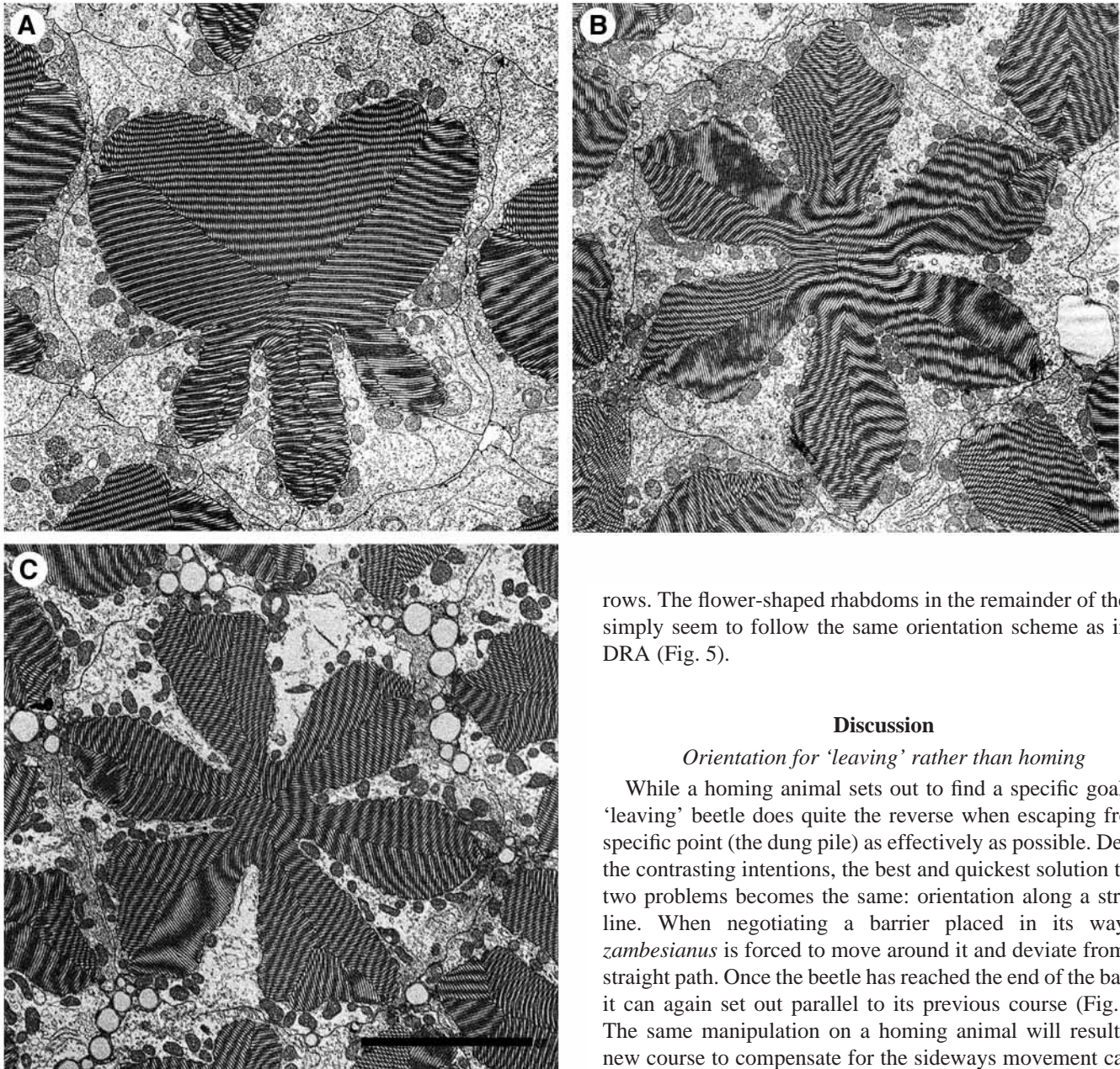


Fig. 4. Electron micrographs of the rhabdoms in the dorsal and ventral eyes. The rhabdoms are formed by seven receptor cells but differ in their shape and microvillar orientation in different eyes and eye regions. The rhabdoms of the dorsal rim area (A) are heart-shaped with orthogonal microvilli, while the rhabdoms in the rest of the dorsal eye (B) and in the ventral eye (C) are flower-shaped with several microvillar orientations. Scale bar: 5 μm .

to one single direction of polarisation (Nilsson et al., 1987; Wehner et al., 1975). The transverse-axis of the DRA rhabdoms, defined by the microvillar direction of photoreceptor cells 2–7, is oriented in a dorsally converging fan-shaped pattern across the eye (Fig. 5). The border of the DRA can easily be found based on the changing appearance of the rhabdoms, but no sharp border can be seen in the orientation of the ommatidial

rows. The flower-shaped rhabdoms in the remainder of the eye simply seem to follow the same orientation scheme as in the DRA (Fig. 5).

Discussion

Orientation for 'leaving' rather than homing

While a homing animal sets out to find a specific goal, the 'leaving' beetle does quite the reverse when escaping from a specific point (the dung pile) as effectively as possible. Despite the contrasting intentions, the best and quickest solution to the two problems becomes the same: orientation along a straight line. When negotiating a barrier placed in its way, *S. zambesianus* is forced to move around it and deviate from this straight path. Once the beetle has reached the end of the barrier, it can again set out parallel to its previous course (Fig. 6A). The same manipulation on a homing animal will result in a new course to compensate for the sideways movement caused by the barrier (Fig. 6B; Frantsevich et al., 1977; Schmidt et al., 1992). The direction of this new course is obtained by path integration (Müller and Wehner, 1988). If the path-integrating animal is instead picked up and transferred sideways, it cannot compensate for the sideways movement and behaves as the ball-rolling beetle (Fig. 6C; Wehner, 1982; Wehner and Wehner, 1986). An obvious difference between homing and leaving is, of course, that the leaving beetle has a set place to start, while the homing animal has a determined place to stop. This simplifies the task of orientation, and a parallel course after negotiating an obstacle will still effectively take the beetle away from the busy dung pile while a homing animal might be hopelessly lost.

Orientation to polarised light

For an animal active shortly after sunset, the visual cues

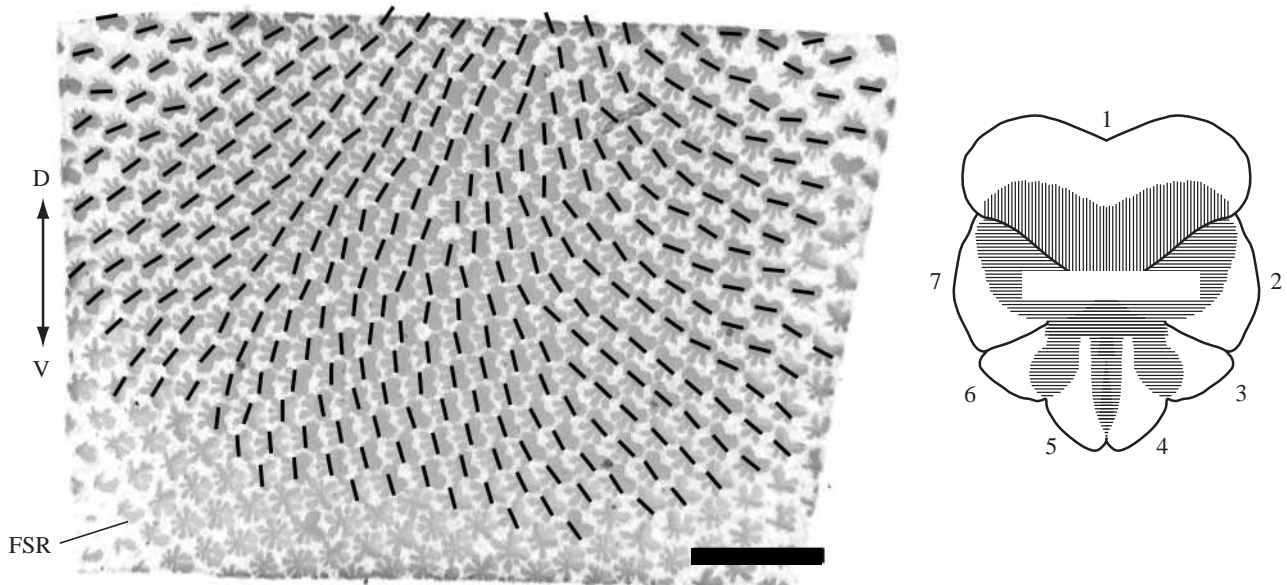


Fig. 5. A light microscopical section through most of the dorsal rim area (DRA). The double-headed arrow marks the orientation of the section (D, dorsal; V, ventral). The black bars, indicating the microvillar orientation of retinula cells 2–7 (see the enlarged cross-section, orientation indicated in this case by a white bar), form a fan-shaped pattern across the DRA. Besides the shape of the rhabdoms, no distinct border in distribution or orientation of the ommatidia indicates the limits of the DRA. The flower-shaped rhabdoms (FSR) in the remainder of the eye are seen at the bottom of the image. Scale bar: 50 μ m.

that can be used for orientation are fairly limited. It is just dark enough for the stars to be visible and the moon is not always present. Terrestrial landmarks might be used but they will be hard to detect at these low light levels. The only cues left are the polarised light pattern present in the sky together with a brighter sky towards the setting sun. When the direction of the pattern of polarised skylight was artificially switched by 90°, the beetle accordingly changed its course. The same response, but less pronounced, can also be observed in the laboratory. The somewhat smaller turn of the beetle in the laboratory is likely to be explained by a decreased desire to roll when kept in captivity. Nevertheless,

both experiments clearly indicate that the beetles perceive the e-vector of light and use it to roll their balls along a chosen path.

In the field, a polarising filter will artificially change the intensity of the skylight polarisation pattern. When the filter is placed with its e-vector transmission axis perpendicular to the dominant polarisation direction of skylight at dusk (north–south), the darkest part of the sky will now be perceived from the zenith (where the degree of polarisation is the highest) rather than from the east. West, however, will still remain the brightest part of the sky and could still serve as a compass cue for orientation. The recorded turn of the beetles under the filter thus shows that polarised light could well be the primary cue used by the beetle to maintain its bearing. The little dance performed on the top of the ball before *S. zambesianus* starts to roll supports this idea. In other navigating insects, such rotations are believed to recalibrate the polarization compass before they start their journey (Wehner, 1997).

A course-stabilising function of polarisation vision has also been suggested in flies (von Philipsborn and Labhart, 1990; Wellington, 1953; Wolf et al., 1980; Wunderer and Smola, 1982) and crickets (Brunner and Labhart, 1987). Common to these insects, and most other polarisation-sensitive insects, is that the receptors used for celestial polarisation analysis are restricted to the DRA of the eye (Labhart

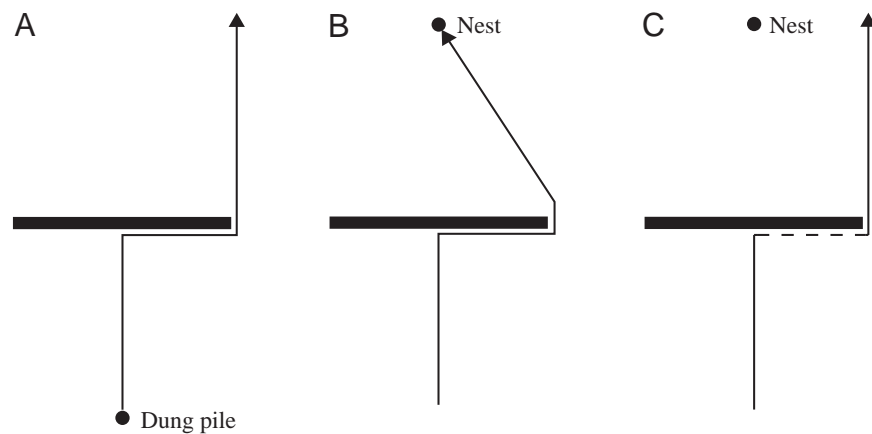


Fig. 6. Schematic drawing of theoretical paths travelled by 'leaving' (A) and homing (B,C) animals when negotiating a barrier (black rectangle). In C, the animal is picked up and displaced sideways (broken line) as it reaches the barrier.

and Meyer, 1999). This also holds true for *S. zambesianus*.

A DRA for polarised light detection

The location of the DRA is revealed by the shape of the rhabdoms. The two sets of receptors with parallel microvilli, oriented 90° to each other, can be found only in the rhabdoms within this dorsal area and these well satisfy the requirements for a polarisation opponent analyzer. A second important characteristic for high polarisation sensitivity is that the microvilli are well aligned along the length of the rhabdom (Nilsson et al., 1987; Wehner et al., 1975).

In some animals, the location of the DRA can be observed from the surface of the eye. Light-scattering cavities in the cornea, or differently shaped facets, discriminate this area from the rest of the eye (Aepli et al., 1985; Burghause, 1979; Labhart et al., 1992; Meyer and Labhart, 1981; Ukhanov et al., 1996). No such differences can be found in the cornea or are visible on the smooth, glassy surface of the eye in *S. zambesianus*; neither has it been reported in the dung beetle *P. striatum* (Dacke et al., 2002) or in any other morphological study of the eyes of dung beetles (Scarabaeidae) where an ability to detect the polarisation of light has been suggested (Gokan, 1989a,b,c, 1990; Gokan and Meyer-Rochow, 1990; Meyer-Rochow, 1978). Light-scattering cavities, or differently shaped facets to aid in polarised light detection, thus appear to be absent within the dung beetles (Scarabaeidae).

Adaptations for polarised light detection at low light levels

For polarised light orientation around sunset, the sensitivity of the detector becomes more and more critical as light levels fall continuously with the setting sun. Low light intensities will become a problem for the whole visual system, but here we focus our interest only on the DRA. The dung beetle *P. striatum* is also able to orient to polarised skylight but is active during the day in one of the brightest habitats on earth: the sun-flooded desert plain (Dacke et al., 2002; Scholtz, 1989). How does the optical sensitivity of the DRA in *S. zambesianus* compare with that in this day-navigating species?

The difference between the two species becomes obvious upon comparison of the rhabdoms from the DRA (Fig. 7; Table 1). The rhabdom in *S. zambesianus* is both much longer and almost three times as wide as that in *P. striatum*. This allows the receptors of *S. zambesianus* to collect more light and, thus, makes them more sensitive (Land, 1981). In addition, the tracheal tapetum of *S. zambesianus* reflects light back through the rhabdom a second time, effectively making the rhabdom twice as long. An estimate of how much more

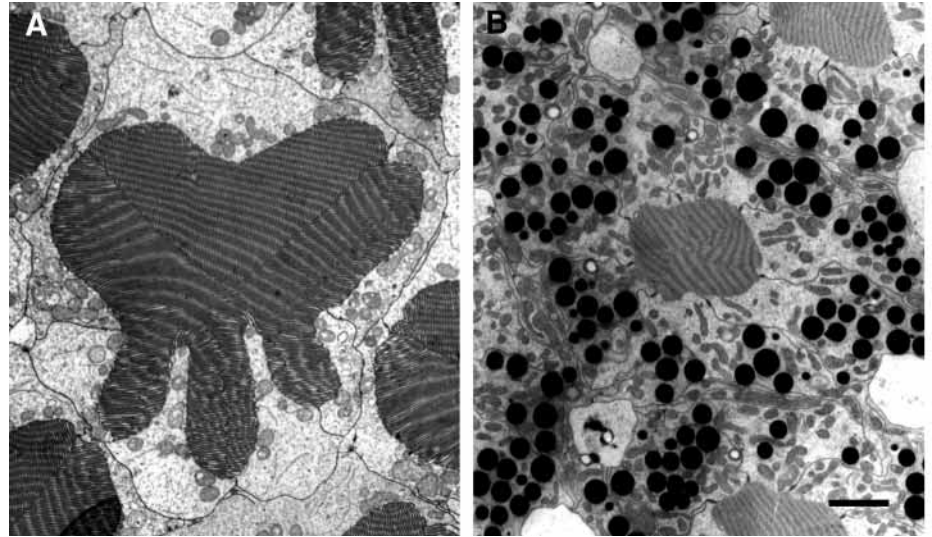


Fig. 7. Cross-sections of DRA-rhabdoms in the crepuscular beetle *Scarabaeus zambesianus* (A) and the diurnal beetle *Pachysoma striatum* (B). Note the difference in the size of the rhabdom and amount of pigmentation, both morphological adaptations to the time of activity. Scale bar: 2 μm .

sensitive the DRA in *S. zambesianus* is can be obtained by calculating its optical sensitivity, S , to an extended source of light (Kirschfeld, 1974; Land, 1981, 1989; modified for white light by Warrant and Nilsson, 1998):

$$S = \left(\frac{\pi}{4}\right)^2 A^2 \left(\frac{d}{f}\right)^2 \left(\frac{kl}{2.3 + kl}\right), \quad (1)$$

where A is the diameter of the superposition aperture, f is the posterior nodal distance of the eye, d and l are the rhabdom diameter and length, respectively, and k is the extinction coefficient of the rhabdom (taken as $0.0067 \mu\text{m}^{-1}$; Bruno et al., 1977). Unfortunately, A is unknown in both species and we will thus be unable to obtain an absolute value of S . However, using values of d , l and f for the two species (given in Table 1), we obtain $S=9.6 \times 10^{-5} A_{sz}^2 \mu\text{m}^2 \text{sr}$ for *S. zambesianus* and $S=1.7 \times 10^{-5} A_{ps}^2 \mu\text{m}^2 \text{sr}$ for *P. striatum*. A_{sz} and A_{ps} are the unknown diameters of the superposition apertures in the two species. Even if $A_{sz}=A_{ps}$, the DRA in *S. zambesianus* is still 5.6 times more sensitive to an extended source of light than is the

Table 1. Dimensions of rhabdoms and focal lengths in the dorsal rim area of two dung beetle species

| Species | d (μm) | l (μm) | f (μm) |
|-------------------------------|--------------------------|--------------------------|--------------------------|
| <i>Scarabaeus zambesianus</i> | 11 | 120 | 555 |
| <i>Pachysoma striatum</i> | 4 | 78 | 328 |

d , rhabdom diameter; l , rhabdom length; f , focal length.

Measurements were taken from electron microscopical sections (d) and light microscopical sections (l, f). d is calculated from the area of the non-circular rhabdoms.

DRA in *P. striatum*. However, in reality, this difference in sensitivity is likely to be much higher. This is because *S. zambesianus* – a nocturnal and crepuscular species with large eyes – can be expected to have a much wider superposition aperture than the diurnal *P. striatum*. The superposition eyes of animals from dim habitats tend to have much larger superposition apertures than those of animals living in bright habitats (McIntyre and Caveney, 1998). Moreover, the rhabdoms in the DRA of *S. zambesianus* are isolated from each other by a reflective tracheal sheath, while those of *P. striatum* are isolated by light-absorbing pigments, a morphological difference that will make the sensitivity difference even greater (Warrant and McIntyre, 1991).

Do the beetles stop foraging when they can no longer perceive and orient to the e-vector of light? At this point, we can only hypothesise about whether this is the case. On a moonless night, the beetles cease their activity 40–50 min after sunset. This halt in activity coincides with the time of night when the light intensity drops dramatically and the degree of polarisation at the sky's zenith decreases from 45% to 5% within 15 min (Dave and Ramanathan, 1956). Crickets can detect the e-vector of strongly polarised light at intensities that are even lower than that from a clear moonless sky, and during the day they need no more than 5% polarisation to detect the direction of polarisation (Herzmann and Labhart, 1989; Labhart, 1996). For *S. zambesianus*, low light intensities will unfortunately coincide with low degrees of polarisation, and the critical threshold for orientation to the polarisation of twilight skylight will thus probably occur at higher intensities and degrees of polarisation than those recorded for crickets. On moonlit nights, the beetles stay active longer than on moonless nights, possibly using the moon as an orientation source when the polarised light pattern from the sun is no longer available.

A fan-shaped pattern of the analysers is a consequence of the ontogeny of the eye

S. zambesianus has the same ommatidial array as that found in the DRA of all diurnal (Burghause, 1979; Dacke et al., 2002; Wehner, 1982; Wunderer and Smola, 1982) and crepuscular (Labhart et al., 1992) animals where this has been carefully mapped. This arrangement is remarkably stable between different insect groups, irrespective of whether they have superposition or apposition eyes. It is also stable between insects orienting to the polarisation of skylight at different times of the day. About 15 years ago, this fan-shaped arrangement of the ommatidial axis in the DRA was proposed to mimic the e-vector pattern of skylight (Wehner 1989), but even then the possibility was raised that the arrangement could be a natural consequence of the spherical shape of the eye. In *S. zambesianus*, we found no morphological difference in the arrangement of the ommatidial rows in the DRA from that in the rest of the eye. This supports the idea that the arrangement of the rhabdoms in the DRA is simply a consequence of the way the eye is built, rather than an adaptation to polarised light analysis. Adaptations for polarised light detection are more likely restricted to the optics of the eye, the orthogonal

arrangement of the microvilli and in the way in which the signals from the polarisation analysers are pooled (Blum and Labhart, 2000; Labhart et al., 2001).

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References

- Aeppli, F., Labhart, T. and Meyer, E. P. (1985). Structural specializations of the cornea and retina at the dorsal rim of the compound eye in hymenopteran insect. *Cell. Tissue Res.* **239**, 19–24.
- Blum, M. and Labhart, T. (2000). Photoreceptor visual fields, ommatidial array, and receptor axon projections in the polarisation-sensitive dorsal rim area of the cricket compound eye. *J. Comp. Physiol. A* **186**, 119–128.
- Brines, M. L. and Gould, J. L. (1982). Skylight polarisation patterns and animal orientation. *J. Exp. Biol.* **96**, 69–91.
- Brunner, D. and Labhart, T. (1987). Behavioural evidence for polarization vision in crickets. *Physiol. Entomol.* **12**, 1–10.
- Bruno, M. S., Barnes, S. N. and Goldsmith, T. H. (1977). The visual pigment and visual cycle of the lobster *Homarus*. *J. Comp. Physiol.* **120**, 123–142.
- Burghause, F. M. H. R. (1979). Die strukturelle Spezialisierung des dorsalen Agentteils der Grillen (Orthoptera, Grylloidea). *Zool. Jb. Physiol.* **83**, 502–525.
- Coemans, M. A. J. M., Vos Hzn, J. J. and Nuboer, J. F. W. (1994). The orientation of the E-vector or linearly polarized light does not affect the behavior of the pigeon *Columbia livia*. *J. Exp. Biol.* **191**, 107–123.
- Dacke, M., Nilsson, D.-E., Warrant, E. J., Blest, A. D., Land, M. F. and O'Carroll, D. C. (1999). Built-in polarizers form part of a compass organ in spiders. *Nature* **401**, 470–473.
- Dacke, M., Doan, T. A. and O'Carroll, D. C. (2001). Polarized light detection in spiders. *J. Exp. Biol.* **204**, 2481–2490.
- Dacke, M., Nordström, P., Scholtz, C. H. and Warrant, E. J. (2002). A specialized dorsal rim area for polarized light detection in the compound eye of the scarab beetle *Pachysoma striatum*. *J. Comp. Physiol. A* **188**, 211–216.
- Dave, J. V. and Ramanathan, K. R. (1956). On the intensity and polarisation of the light from the sky during twilight. *Proc. Indian Acad. Sci.* **43A**, 67–68.
- Frantsevich, L., Govardovsky, V., Gribakin, F., Nikolajev, G., Pichka, V., Polanovsky, A., Shevchenko, V. and Zolotov, V. (1977). Astroorientation in *Lethrus* (Coleoptera, Scarabaeidae). *J. Comp. Physiol.* **121**, 253–271.
- Gál, J., Horváth, G., Barta, A. and Wehner, R. (2001). Polarization of the moonlit clear night sky measured by full-sky imaging polarimetry at full moon: comparison of the polarization of moonlit and sunlit skies. *J. Geophys. Res.* **106**, 22647–22653.
- Gokan, N. (1989a). Fine structure of the compound eye of the dung beetle *Ochodaeus maculatus* (Coleoptera, Scarabaeidae). *Jpn. J. Ent.* **57**, 823–830.
- Gokan, N. (1989b). Fine structure of the compound eye of the dung beetle *Aphodius haroldianus* (Coleoptera: Scarabaeidae). *Appl. Ent. Zool.* **24**, 483–486.
- Gokan, N. (1989c). The compound eye of the dung beetle *Geotrupes auratus* (Coleoptera: Scarabaeidae). *Appl. Ent. Zool.* **24**, 133–146.
- Gokan, N. (1990). Fine structure of the compound eye of the dung beetle *Onthophagus lenzii* (Coleoptera, Scarabaeidae). *Jpn. J. Ent.* **58**, 185–195.
- Gokan, N. and Meyer-Rochow, V. B. (1990). The compound eye of the dung beetle, *Onthophagus posticus* (Coleoptera: Scarabaeidae). *N. Z. Ent.* **13**, 7–15.
- Goldsmith, T. H. and Wehner, R. (1977). Restrictions on rotational and translational diffusion of pigment in the membranes of a rhabdomeric photoreceptor. *J. Gen. Physiol.* **70**, 453–490.
- Hanski, I. and Cambefort, Y. (1991). *Dung Beetle Ecology*. Princeton, NJ: Princeton University Press.
- Hardie, R. C. (1984). Properties of photoreceptors R7 and R8 in dorsal marginal ommatidia in the compound eyes of *Musca* and *Calliphora*. *J. Comp. Physiol. A* **154**, 157–165.

- Helbig, A. J.** (1990). Depolarization of natural skylight disrupts orientation of an avian nocturnal migrant. *Experientia* **46**, 755-758.
- Helbig, A. J.** (1991). Dusk orientation of migratory European robins, *Erithacus rubecula*: the role of sunrelated directional information. *Anim. Behav.* **41**, 313-322.
- Herzmann, D. and Labhart, T.** (1989). Spectral sensitivity and absolute threshold of polarization vision in crickets: a behavioral study. *J. Comp. Physiol. A* **165**, 315-319.
- Israelachvili, J. N. and Wilson, M.** (1976). Absorption characteristics of oriented photopigments in microvilli. *Biol. Cybern.* **21**, 9-15.
- Kirschfeld, K.** (1974). The absolute sensitivity of lens and compound eyes. *Z. Naturforsch. (C)* **29**, 592-596.
- Labhart, T.** (1980). Specialized photoreceptors at the dorsal rim of the honeybee's compound eye: polarizational and angular sensitivity. *J. Comp. Physiol.* **141**, 19-30.
- Labhart, T.** (1988). Polarization-opponent interneurons in the insect visual system. *Nature* **331**, 435-437.
- Labhart, T.** (1996). How polarization-sensitive interneurons of crickets perform at low degrees of polarization. *J. Exp. Biol.* **199**, 1467-1475.
- Labhart, T. and Meyer, E. P.** (1999). Detectors for polarized skylight in insects: a survey of ommatidial specializations in the dorsal rim area of the compound eye. *Microsc. Res. Tech.* **47**, 368-379.
- Labhart, T., Meyer, E. P. and Schenker, L.** (1992). Specialized ommatidia for polarization vision in the compound eye of cockchafers, *Melolontha melolontha* (Coleoptera, Scarabaeidae). *Cell. Tissue Res.* **268**, 419-429.
- Labhart, T., Petzold, P. and Helbling, H.** (2001). Spatial integration in polarization-sensitive interneurons of crickets: a survey of evidence, mechanisms and benefits. *J. Exp. Biol.* **204**, 2423-2430.
- Land, M. F.** (1981). Optics and vision in invertebrates. In *Handbook of Sensory Physiology*, vol. VII/6B (ed. H. Autrum), pp. 471-592. Berlin, Heidelberg, New York: Springer Verlag.
- Land, M. F.** (1989). Variations in the structure and design of compound eyes. In *Facets of Vision* (ed. D. G. Stavenga and R. C. Hardie), pp. 90-111. Berlin, Heidelberg: Springer Verlag.
- McIntyre, P. D. and Caveney, S.** (1998). Superposition optics and the time of flight in onitine dung beetles. *J. Comp. Physiol. A* **183**, 45-60.
- Meyer, E. P. and Labhart, T.** (1981). Pore canals in the cornea of a functionally specialized area of the honey bee's compound eye. *Cell. Tissue Res.* **216**, 491-501.
- Meyer, E. P. and Labhart, T.** (1993). Morphological specializations of dorsal rim ommatidia in the compound eye of dragonflies and damselflies (Odonata). *Cell. Tissue Res.* **272**, 17-22.
- Meyer-Rochow, V. B.** (1978). Retina and dioptic apparatus of the dung beetle *Euonticellus africanus*. *J. Insect. Physiol.* **24**, 165-179.
- Moore, F. R. and Phillips, J. B.** (1988). Sunset, skylight polarization and the migratory orientation of yellowrumped warblers, *Dendroica coronata*. *Anim. Behav.* **36**, 1770-1778.
- Müller, M. and Wehner, R.** (1988). Path integration in desert ants, *Cataglyphis fortis*. *Proc. Natl. Acad. Sci. USA* **85**, 5287-5290.
- Nilsson, D.-E. and Warrant, E. J.** (1999). Visual discrimination: seeing the third quality of light. *Curr. Biol.* **9**, R535-R537.
- Nilsson, D., Labhart, T. and Meyer, E. P.** (1987). Photoreceptor design and optical properties affecting polarization sensitivity in ants and crickets. *J. Comp. Physiol. A* **161**, 645-658.
- Phillips, J. B. and Moore, F. R.** (1992). Calibration of the sun compass by sunset polarized light patterns in a migratory bird. *Behav. Ecol. Sociobiol.* **31**, 189-193.
- Rozenberg, G. V.** (1966). *Twilight: a Study in Atmospheric Optics*. New York: Plenum Press.
- Schmidt, I., Collett, T. S., Dillier, F.-X. and Wehner, R.** (1992). How desert ants cope with enforced detours on their way home. *J. Comp. Physiol. A* **171**, 285-288.
- Scholtz, C. H.** (1989). Unique foraging behaviour in *Pachysoma* (= *Scarabaeus striatum* Castelnau (Coleoptera:Scarabaeidae): an adaptation to arid conditions? *J. Arid Env.* **16**, 305-313.
- Ukhanov, K., Leertouwer, H. L., Gribakin, F. G. and Stavenga, D. G.** (1996). Dioptics of the facet lenses in the dorsal rim area of the cricket *Gryllus bimaculatus*. *J. Comp. Physiol. A* **179**, 545-552.
- von Philipsborn, A. and Labhart, T.** (1990). A behavioural study of polarization vision in the fly, *Musca domestica*. *J. Comp. Physiol. A* **167**, 737-743.
- Warrant, E. J. and McIntyre, P. D.** (1991). Strategies for retinal design in arthropod eyes of low F-number. *J. Comp. Physiol. A* **168**, 499-512.
- Warrant, E. J. and Nilsson, D.-E.** (1998). Absorption in of white light in photoreceptors. *Vision Res.* **38**, 195-207.
- Waterman, T. H.** (1981). Polarization sensitivity. In *Handbook of Sensory Physiology*, vol. VII/6B (ed. H. Autrum), pp. 281-469. Berlin, Heidelberg, New York: Springer Verlag.
- Wehner, R.** (1982). Himmelsnavigation bei Insekten. *Neurophysiologie und Verhalten. Neujahrsbl. Naturforsch. Ges. Zürich* **184**, 1-132.
- Wehner, R.** (1989). The hymenopteran skylight compass: matched filtering and parallel coding. *J. Exp. Biol.* **146**, 63-85.
- Wehner, R.** (1997). The ant's celestial compass system: spectral and polarization channels. In *Orientation and Communication in Arthropods* (ed. M. Lehrer), pp. 145-185. Basel: Birkhäuser Verlag.
- Wehner, R. and Wehner, S.** (1986). Path integration in desert ants. Approaching a long standing puzzle in insect navigation. *Monitore Zool. Ital.* **20**, 309-331.
- Wehner, R., Bernard, G. D. and Geiger, E.** (1975). Twisted and non-twisted rhabdoms and their significance for polarization detection in the bee. *J. Comp. Physiol.* **104**, 225-245.
- Wellington, W. G.** (1953). Motor responses evoked by the dorsal ocelli of *Sarcophaga aldrichi* Parker, and the orientation of the fly to plane polarized light. *Nature* **172**, 1177-1179.
- Wellington, W. G.** (1974). Bumblebee ocelli and navigation at dusk. *Science* **183**, 550-551.
- Wolf, R., Gerbhardt, B., Gademann, R. and Heisenberg, M.** (1980). Polarization sensitivity of course control in *Drosophila melanogaster*. *J. Comp. Physiol.* **139**, 177-191.
- Wunderer, H. and Smola, U.** (1982). Fine structure of ommatidia at the dorsal eye margin of *Calliphora erythrocephala* Meigen (Diptera: Calliphoridae): An eye region specialised for the detection of polarized light. *Int. J. Insect Morphol. Embryol.* **11**, 25-38.