

Julia Stalleicken · Thomas Labhart · Henrik Mouritsen

## Physiological characterization of the compound eye in monarch butterflies with focus on the dorsal rim area

Received: 19 September 2005 / Revised: 21 October 2005 / Accepted: 24 October 2005 / Published online: 30 November 2005  
© Springer-Verlag 2005

**Abstract** The spectral, angular and polarization sensitivities of photoreceptors in the compound eye of the monarch butterfly (*Danaus plexippus*) are examined using electrophysiological methods. Intracellular recordings reveal a spectrally homogenous population of UV receptors with optical axes directed upwards and  $\geq 10^\circ$  to the contralateral side. Based on optical considerations and on the opsin expression pattern (Sauman et al. 2005), we conclude that these UV receptors belong to the anatomically specialized dorsal rim area (DRA) of the eye. Photoreceptors in the main retina with optical axes  $< 10^\circ$  contralateral or ipsilateral have maximal sensitivities in the UV ( $\lambda_{\max} \leq 340$  nm), the blue ( $\lambda_{\max} = 435$  nm) or in the long-wave range (green,  $\lambda_{\max} = 540$  nm). The polarization sensitivity (PS) of the UV receptors in the DRA is much higher (PS = 9.4) than in the UV cells (PS = 2.9) or green cells (PS = 2.8) of the main retina. The physiological properties of the photoreceptors in the DRA and in the main retina fit closely with the anatomy and the opsin expression patterns described in these eye regions. The data are discussed in the light of present knowledge about polarized skylight navigation in Lepidopterans.

**Keywords** *Danaus plexippus* · Compound eye · Dorsal rim area · Polarization sensitivity · Spectral sensitivity

**Abbreviations** DA: Dorsal area · DRA: Dorsal rim area · ERG: Electroretinogram · PS: Polarization sensitivity

### Introduction

As a result of sunlight scattering in the atmosphere, skylight is partly plane polarized. Insects can exploit skylight polarization for different orientation tasks, for example for course control as observed in flies (Wolf et al. 1980; von Philipsborn and Labhart 1990), or spatial orientation in the context of foraging and homing as observed in bees and ants (Wehner 1984, 1994, 1996). It has also been hypothesized that migratory insects such as locusts (Mappes and Homberg 2004, Homberg 2004) or migratory lepidopterans may use the pattern of polarized skylight as a global reference cue for their compass systems. Whether or not this is the case in long-distance migratory monarch butterflies (*Danaus plexippus*) during their annual autumn migration is a matter of debate (Reppert et al. 2004; Saumann et al. 2005; Stalleicken et al. 2005). In insects, the perception of the e-vector orientation in polarized skylight is mediated by anatomically and physiologically specialized ommatidia located at the dorsal margin of the eye, the dorsal rim area (DRA) (for reviews, see Labhart and Meyer 1999; Wehner and Labhart 2005, in press). The DRA of monarch butterflies consists of approximately 100 anatomically specialized ommatidia forming a narrow ribbon, which is maximally three ommatidia wide. These ommatidia show the typical features associated with polarized light detection such as wide and short rhabdoms, two types of photoreceptors with orthogonal microvilli orientation and strictly aligned microvilli per photoreceptor (Labhart and Meyer 1999; Labhart and Baumann 2003; Reppert et al. 2004; Saumann et al. 2005). Microvillar alignment in combination with short rhabdomeres suggests that the photoreceptors in the monarch DRA are highly polarization sensitive (Nilsson et al. 1987). Furthermore, the polarization sensors in the DRA should receive monochromatic input in order to avoid interference between e-vector and wavelength information (Labhart and Meyer 1999; Wehner and Labhart 2005, in press). The spectral sensitivity of a

J. Stalleicken (✉) · H. Mouritsen  
VW Nachwuchsgruppe Animal Navigation, IBU,  
University of Oldenburg, 26111 Oldenburg, Germany  
E-mail: julia.stalleicken@uni-oldenburg.de  
Tel.: +49-441-7983095

T. Labhart  
Zoological Institute, University of Zürich,  
Winterthurerstrasse 190, 8057 Zürich, Switzerland

photoreceptor depends mainly on the absorbance spectrum of the visual pigment in the microvillar membranes. The absorbance spectrum, in turn, is mainly determined by the opsin component of the visual pigment. Saumann et al. (2005) studied the distribution of UV, blue and green (long-wave) opsin expressed by the photoreceptor cells in the monarch retina. They found that the photoreceptors in the DRA express only UV opsin, whereas in other parts of the retina UV, blue and long-wave opsins are expressed. The aim of the current study is to measure electrophysiologically the spectral, angular and polarization sensitivities of photoreceptors in the DRA and main retina of the monarch butterfly's compound eye and, thereby to test whether their physiological properties correspond to the anatomical findings and the opsin expression patterns.

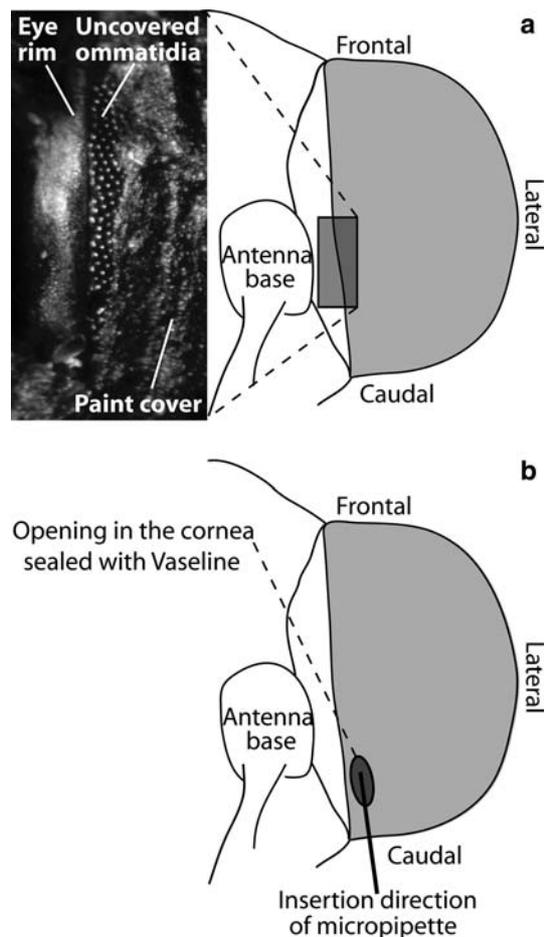
## Methods

### Animals

Pupae of the monarch butterfly (*D. plexippus*) were purchased from London Pupae Supplies (Oxford, UK). After eclosion, the adults were kept at 27°C and 70% relative humidity under long day conditions ( $L/D = 14/10$  h). Lighting was provided by Osram L20W/10S daylight lamps.

### Stimulation

The photoreceptors of the monarch compound eye were studied by using the electroretinogram (ERG) and intracellular recording techniques. For ERG recordings light was provided by a 450 W xenon arc lamp. Quasi-monochromatic light was produced by eleven narrow-band interference filters ranging from 339 to 664 nm. Light intensity was adjusted by a set of neutral density filters. The light was focused into a flexible UV-transmitting light guide providing a stimulus of 17° for a monarch mounted at the other end of it. In order to stimulate selectively ommatidia in different parts of the main retina (dorsal, medial, ventral) the orientation of the eye relative to the stimulus was appropriately adjusted by exploiting the eye glow under orthodromic illumination. For selective stimulation of the DRA, the eye was illuminated from above while the dorsal part of the eye was covered with opaque black paint (Herbol GmbH) except for a narrow window near the approximate center of the DRA (Fig. 1a). If only a small number of facets were illuminated, the ERG signals were too small. Therefore, we had to keep the window 3–5 ommatidia wide and 20–30 ommatidia long and in most cases the window was slightly wider than the DRA. Spectral sensitivity was assessed by adjusting the intensity of 100 ms monochromatic flashes such that the amplitude of the ERG-response was the same for all wavelengths.



**Fig. 1** Recording methodology (top view onto the right hemisphere of the monarch head). **a** For ERG recordings from the dorsal rim area (DRA), the dorsal part of the eye was covered with black paint except for a narrow window at the approximate center of the DRA (next to the antenna base; position indicated by shaded box). **b** For intracellular recordings, a micropipette was inserted through a small opening in the cornea (dark grey oval) caudal to the antenna base and close to the eye rim

For the intracellular recordings light was provided by a 900 W xenon arc lamp. Quasi-monochromatic light stimuli were generated by 16 narrow band interference filters ranging from 341 to 641 nm. Light intensity was adjusted by a neutral-density wedge and an electromagnetic shutter provided temporal control of the stimulus. The light was focused into a flexible UV-transmitting light-guide with its other end mounted on a perimeter device. A linear polarizer (HNP'B, Polaroid Company) could be inserted between the light-guide and the eye of the butterfly. The stimulus had a diameter of 1° and a duration of 100 ms; the interstimulus interval was 5 or 10 s. When a photoreceptor was impaled, the stimulus was positioned approximately at the center of the receptor's visual field (maximal response amplitude). Spectral sensitivity was determined by successive monochromatic flashes of different wavelengths. The intensity of the stimuli was adjusted to equal quanta and

was periodically checked with a calibrated photodiode (PIN-10-UV, United Detector Technology). The angular sensitivity of a photoreceptor was measured with series of light flashes moving stepwise by  $1^\circ$  on two orthogonal ( $x$ - and  $y$ -) axes, thereby scanning the visual field. To assess PS, the inserted polarizer (and therefore the  $e$ -vector orientation) was turned in steps of  $18^\circ$  until a full  $360^\circ$  turn was completed.  $R/\log I$  functions (calibration curves needed to convert receptor responses to sensitivities) were taken between spectral, polarization and angular scans by applying 0.33 log intensity series of flashes. Angular and polarization sensitivities as well as  $R/\log I$  functions were measured at the wavelength matching the highest spectral sensitivity of the recorded photoreceptor. Occasionally, during experiments with less sensitive cells, a K36 (Schott) broadband UV filter was used instead of a UV-interference filter.

### Preparation and recording

The monarchs were immobilized and mounted on a holder with wax. For ERG recordings an electrolytically sharpened tungsten electrode was inserted into the eye, while the reference electrode was placed in the antenna base. The extracellular signals were preamplified (Model P15, Grass Instruments) and monitored with a storage oscilloscope. For intracellular recordings a small piece of the cornea and the underlying crystalline cones were removed with a razor blade fragment close to the rim of the eye and caudal to the antenna base (Fig. 1b). To prevent desiccation, the opening was immediately sealed with Vaseline using a warm heating coil. After orienting the head for suitable electrode approach the animal was mounted in the perimeter with the right eye in the center. Glass micropipettes filled with 2 mol/l KCL (resistance 100–150 M $\Omega$ ) were introduced into the eye through the opening and advanced in frontal direction. The reference electrode consisted of a sharpened platinum wire and was placed in the butterfly's abdomen. Intracellular signals from photoreceptors were measured using a high-impedance electrometer (M707, World Precision Instruments) and monitored on a storage oscilloscope. Cell responses, polarizer orientation and shutter state were recorded on a chart recorder (Model 8830, Hioki corporation).

### Evaluation of data

For the ERG recordings, spectral sensitivities were calculated as the reciprocal values of the light intensities that produced a specific ERG amplitude at all wavelengths. Intracellular spectral, angular and polarization sensitivities were calculated from the corresponding response functions and the corresponding  $R$ -log  $I$  functions. Data were evaluated from stable recordings only, that showed short depolarizations to the 100 ms flashes exhibiting neither afterpotentials nor hyperpolarizing phases. No

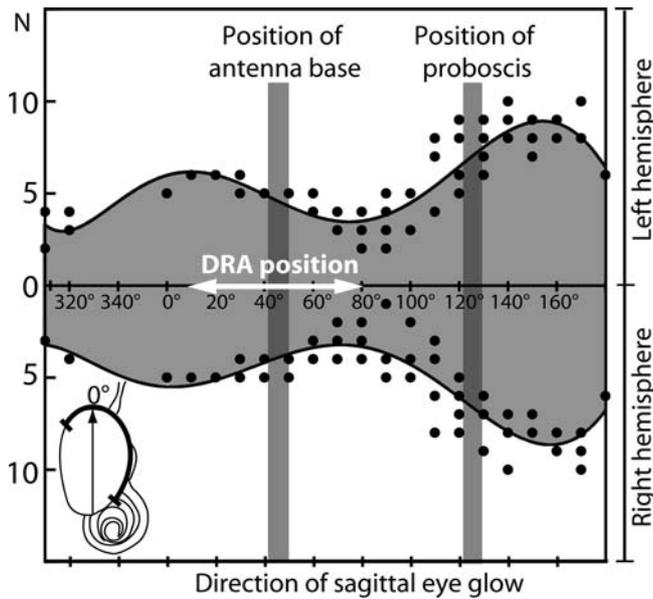
baseline shift was allowed (1) during measurements of response functions and (2) between measurements of spectral, angular or polarization response functions and the corresponding  $R/\log I$  functions (measured in short succession after one another). The acceptance angle of a photoreceptor is defined as the half-width of the angular sensitivity function. To determine acceptance angles, angular scans in both  $x$ - and  $y$ -direction should pass through the center of the visual field, i.e., in both scans maximal response should occur at the same perimeter coordinates. Therefore, no acceptance angles were calculated for scans missing the visual field center by an angle corresponding to  $<80\%$  sensitivity (see Blum and Labhart 2000). Since the stimulus did not move on a great circle during the  $x$ -angular sensitivity measurements, the perimeter readings had to be corrected (Burkhardt and Streck 1965). PS is defined as the ratio between maximal and minimal sensitivity of the photoreceptor to  $e$ -vector orientation. To allow for a later assignment of a given photoreceptor to either the main retina or the DRA, the optical axis of each receptor was determined from (1) the perimeter coordinates of the stimulus for maximal response, (2) the orientation of the head with respect to the perimeter system and (3) the assumed natural head posture (pitch) of flying monarchs. Exact measurements of head posture in free flight do not exist. However, a reasonable estimate is a head pitch for which the long axis of the eye (as seen from lateral) is oriented vertically.

---

## Results

### Optical axes in the dorsal part of the eye

The ommatidia in the main retina of the monarch exhibit a bright orange-red eye glow (for pictures of typical eye glow patterns in nymphalid butterflies, see Briscoe and Bernard 2005), whereas no eye glow could be observed in the DRA. This is probably caused by a gap of approximately 50  $\mu\text{m}$  separating the proximal tip of the rhabdom and the distal end of the light reflecting tapetum (T. Labhart, unpublished). Furthermore, the observation of an antidromic pseudopupil is prohibited by the strong pigmentation of the basal membrane. Thus, in monarchs, the optical axes of the DRA ommatidia cannot be determined directly by an optical method. However, an eye glow can easily be observed in the ommatidia marking the borderline between ipsi- and contralaterally directed ommatidia, i.e., the ommatidia located at the sagittal equator of the eye. Near the approximate center of the DRA, we counted four to five facets (ommatidia) between the sagittal eye glow and the eye rim (Fig. 2). At this position, the DRA reaches the maximal width of three ommatida (Labhart and Bauman 2003), suggesting that the optical axes of DRA ommatidia are directed to the contralateral side. As shown below, the electrophysiological data confirm this view. Note that the optical axes of most of the recorded photoreceptors are in the dorso-frontal rather than

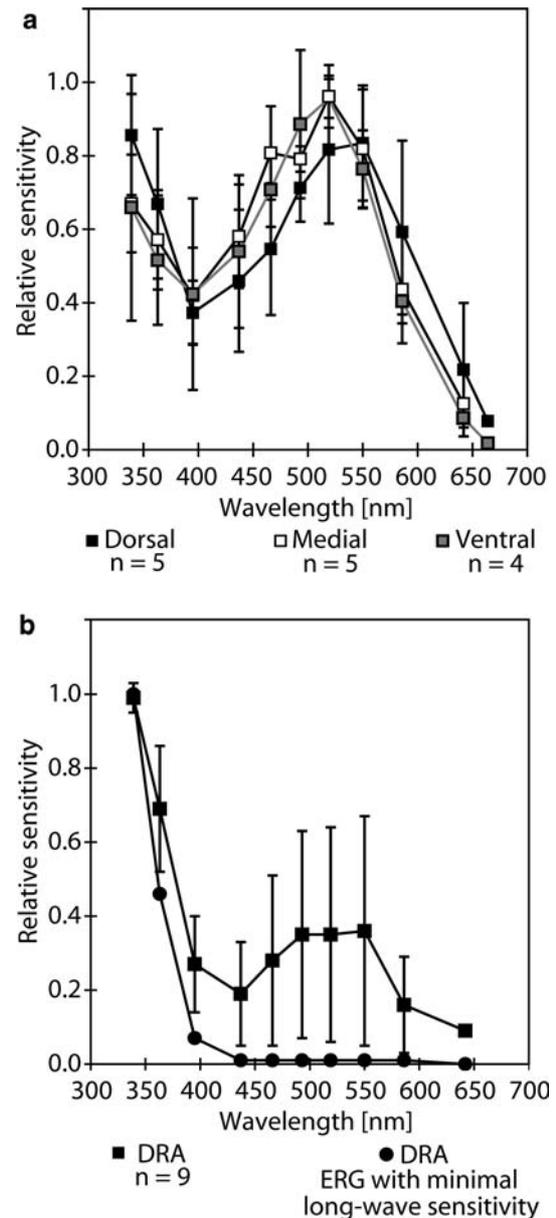


**Fig. 2** Number of facets (ommatidia) between sagittal equator and eye rim. Eye glow in ommatidia having their optical axes in a sagittal plane was observed in four monarchs and the number of facets to the eye rim was counted in both eyes (black dots). The white double arrow indicates the approximate angular position of the DRA as estimated from sagittal eye glow and the anatomical position of the DRA. The inset shows the assumed head pitch in flight (eye long axis vertical as depicted) and indicates the angular range studied (black line around eye). The comparison of the anatomical maximal width of the DRA (three ommatidia) and the number of facets between sagittal eye glow and eye rim (four to five facets) suggests that the ommatidia of the DRA are directed contralaterally

in the dorsal visual field (Fig. 5). This is because the position of the opening in the cornea, through which the electrode was advanced in a frontal direction, excluded recordings from dorsal-caudal parts of the dorsal retina.

### Spectral sensitivity

The spectral sensitivity curves derived from ERG recordings represent the overall spectral sensitivity of several ommatidia and therefore many receptor cells. In the main retina, the spectral sensitivity curves showed two distinct maxima, one in the long-wave range and one in the UV range, divided by a sensitivity minimum at approximately 400 nm (Fig. 3a). The spectral sensitivities of the ERG as recorded in the four individuals did not differ significantly for the dorsal, medial and ventral part of the compound eye (repeated measures ANOVA with Greenhouse–Geisser correction,  $F_{18,54} = 3.437$ ,  $P = 0.08$ ). In the recordings from a narrow window at the position of the DRA, the average spectral sensitivity curve of nine individuals showed a prominent UV peak. The spectral sensitivity in the long-wave range was strongly decreased but varied considerably between individual preparations. In some cases sensitivity in the

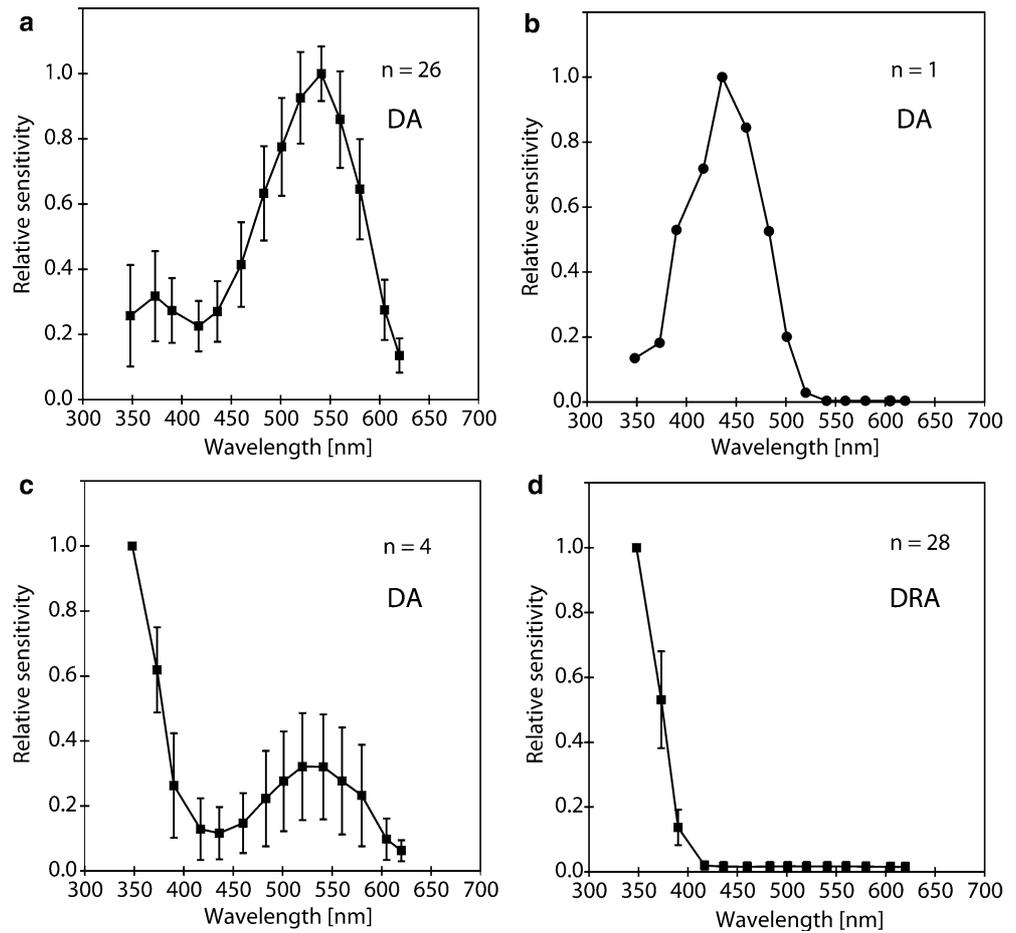


**Fig. 3** Average spectral sensitivity curves derived from ERG-recordings. **a** Measurements from the dorsal, medial and ventral part of the monarch main retina. Measurements were taken from the right eyes of four to five monarchs. **b** Average spectral sensitivity curve derived from ERG recordings in the DRA. Measurements were taken from the right eye of nine monarchs. The spectral sensitivity in the long wavelength part of the spectrum varied considerably between measurements. In some cases it was restricted to the UV range. Squares with error bars in **a** and **b** indicate average sensitivities  $\pm$  SD. Circles in **b** indicate the sensitivity of a recording in the DRA with almost no long-wave response.  $n$  number of measurements

visible range was virtually missing indicating that almost all photoreceptors of the ommatidia illuminated in these experiments were UV receptors (Fig. 3b).

Using intracellular recordings we found three classes of spectral receptors in the retina of the monarch butterfly with maximal sensitivities at  $\lambda_{\max} \leq 340$  nm (UV

**Fig. 4** Average spectral sensitivity curves of different photoreceptor types found in the monarch. **a** Green receptors, **b** one blue receptor and **c** UV receptors were recorded in the dorsal area (DA) of the main retina. The average sensitivity function of the UV receptors in the DA shows a secondary maximum in the long wavelength range that arises most likely from artifactual electric coupling with the predominating green receptors. **d** The average spectral sensitivity function of UV receptors of the monochromatic DRA is missing a secondary green peak. Squares with error bars in **a**, **b** and **d** give average sensitivities  $\pm$ SD. Circles in **b** indicate individual sensitivity values of a single blue receptor. *n* number of recorded cells



receptors), at  $\lambda_{\max}=435$  nm (blue receptors) and at  $\lambda_{\max}=540$  nm (green or long-wave receptors) (see Fig. 4a–c). Cells with optical axes in the ipsilateral and the near contralateral visual field ( $<10^\circ$  contralateral) were predominantly green receptors. UV and blue cells occurred much less frequently (Fig. 5). The green cells tended to show a secondary maximum in the UV. This secondary maximum arises from the  $\beta$ -absorbance of the green visual pigment (Stavenga et al. 1993) and (for UV sensitivities  $>23\%$ ) from artifactual interreceptor coupling with UV receptors as discussed by Labhart (1980). Accordingly, the secondary long-wave maxima observed in the UV receptors of the DA probably arise from artifactual coupling with the prevalent green receptors. Cells with more contralaterally directed optical axes formed a spectrally homogenous population of UV receptors (approximately  $10^\circ$ – $20^\circ$  contralateral; Fig. 5). These receptors missed a secondary maximum in the green range (Fig. 4d). At long wavelengths, most (77%) of these UV cells did not respond to light stimuli, six cells showed very small responses corresponding to maximally 0.8–3% relative sensitivity (for discussion, see below). Our findings are consistent with the results of Sauman et al. (2005) showing that in the monarch DRA all eight main photoreceptor cells per ommatidium express only UV-opsin. In addition, our optical study

(Fig. 2) predicts contralateral optical axes for the ommatidia of the DRA. We, therefore, regard UV receptors with (1) missing or insignificant long-wave sensitivity and (2)  $\geq 10^\circ$  contralaterally directed optical axes as photoreceptors of the DRA.

#### Polarization sensitivity

UV receptors of the DRA showed a high average polarization sensitivity ( $\overline{PS}$ ) of  $9.4 \pm 4.1$  ( $\pm$ SD,  $n=29$ ), although the PS values of individual cells varied considerably (Fig. 6a). The average PS of the UV and green receptors in the DA was much lower ( $\overline{PS} = 2.9 \pm 1.4$ ,  $n=4$  and  $\overline{PS} = 2.8 \pm 0.9$ ,  $n=22$ , respectively; Fig. 6b, c). The PS values of green and UV receptors in the DA differed significantly from those of the UV receptors in the DRA ( $P < 0.001$ , Mann–Whitney test on ranks).

#### Angular sensitivity

In both the DRA and the DA, the visual fields appear to be symmetrically shaped since scans in the  $x$ -direction (approximately orthogonal to the eye rim; DRA:  $n=8$ ; DA:  $n=11$ , Fig. 7a, c) and in the  $y$ -direction (approx-

mately parallel to the eye rim; DRA:  $n=9$ ; DA:  $n=8$ , Fig. 7b, d) show no statistical difference (DRA:  $P=0.61$ , DA:  $P=0.49$ ; Mann–Whitney test on ranks). Therefore, the acceptance angles (half-widths of angular sensitivity functions) for  $x$ - and  $y$ -scans were pooled. In the DRA, the average acceptance angle was  $4.0^\circ \pm 1.1^\circ$  ( $\pm$ SD;  $n=17$ ), in the DA it was  $2.6^\circ \pm 0.5^\circ$  ( $n=19$ ). Thus, the visual fields of DRA ommatidia are slightly wider than those of DA ommatidia ( $P < 0.001$ , Mann–Whitney test on ranks).

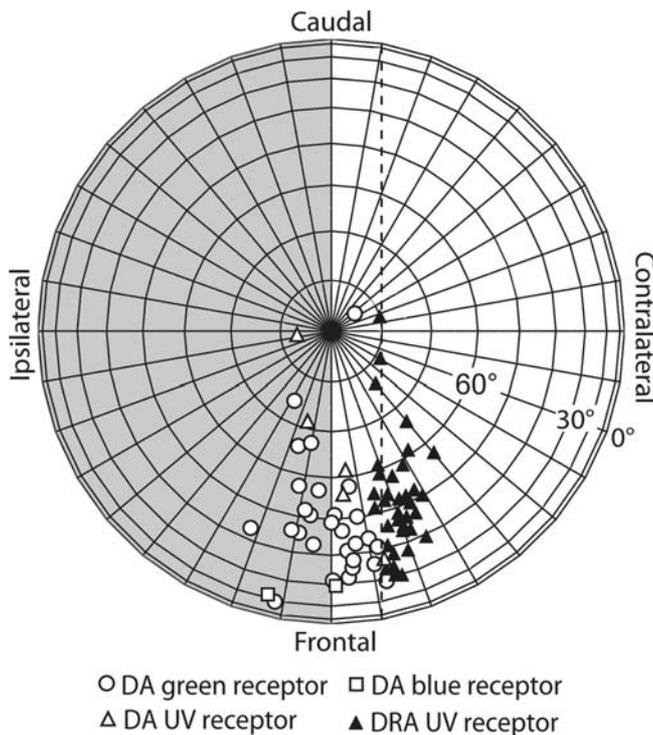
## Discussion

### Spectral sensitivity

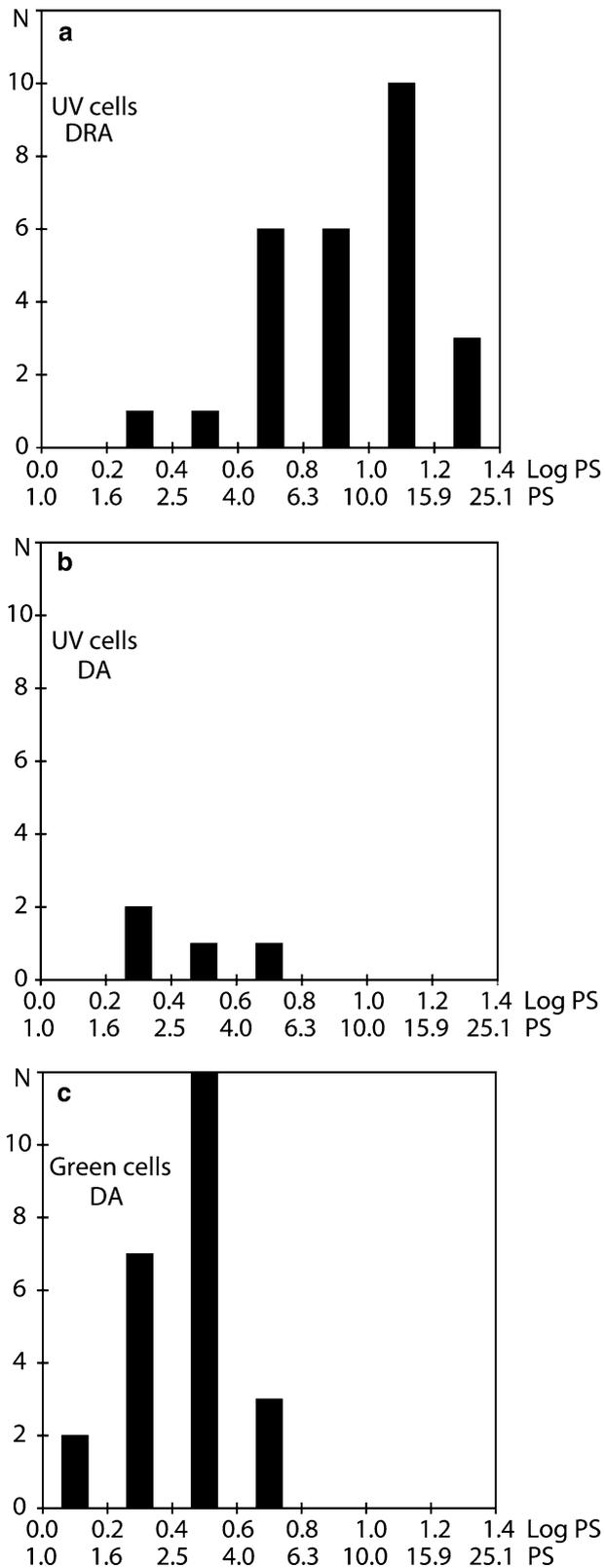
Our electrophysiological findings are consistent with the expression pattern of the visual pigments in the monarch retina as described by Sauman et al. (2005). Intracellular

recordings revealed three spectral classes of photoreceptors in the DA of the monarch eye. Green receptors occurred much more frequently than UV or blue receptors. The predominance of green receptors is in accordance with in-situ hybridization data showing that six out of eight main receptor cells per ommatidium and the small, proximal cell R9 express long-wave opsin, while two receptors individually express either UV or blue opsin (Sauman et al. 2005). A similar opsin expression pattern has been found in another nymphalid butterfly, *Vanessa cardui* (Briscoe et al. 2003). However, the main retina of *V. cardui*, the painted lady, shows a distinct dorso-ventral gradient in UV and blue opsin expression, which is not observed in the monarch retina (Sauman et al. 2005). In the present study, we found that the ERG-derived spectral sensitivity curves of ommatidia in the dorsal, medial or ventral part of the monarch retina do not differ significantly from each other. This suggests that UV and blue receptors are distributed uniformly along the dorso-ventral axis of the retina, which is in agreement with the opsin expression results of Sauman et al. (2005). The characteristics of the spectral sensitivity curves determined with ERG in the main retina (maxima in the UV and in the long-wave range separated by a minimum around 400 nm) are in agreement with the three spectral classes of photoreceptors identified by intracellular recordings. While the contribution of UV and green receptors to the spectral sensitivity curves is immediately apparent, the blue receptors do not produce a separate peak. However, compared to the sensitivity maximum of the green receptors, the long-wave maximum of the ERG is slightly shifted to shorter wavelengths. The spectral sensitivity curves show a striking similarity with those found by Eguchi et al. (1981) in the chestnut tiger, *Parantica sita*, a migratory milkweed butterfly (subfamily Danainae) from Central and East Asia.

Most intracellular recordings indicate that the DRA of the monarch butterfly is strictly monochromatic and sensitive to UV light only. This is consistent with the finding of Sauman et al. (2005) that all the eight main photoreceptors cells express only UV opsin in DRA ommatidia. A few UV cells also gave minute responses to long wavelengths. These could be explained by artifactual coupling to the proximal receptor R9 if this cell contained a long-wave visual pigment. However, Sauman et al. (2005) could not establish whether the proximal receptor R9 expresses any opsin. In addition, no microvilli could be detected in R9 (T. Labhart, unpublished). A contribution of the proximal ninth cells seems, therefore, unlikely. The minute long-wave sensitivity in some UV receptors in the DRA may be due to extracellular effects of DA green receptors, but in fact, the phenomenon remains largely unexplained. The spectral sensitivity curves derived from ERG in the DRA region all peak in the UV. Most ERG recordings focusing on the DRA also showed responses in the long-wave range. This is because in most cases the window in the paint cover of the dorsal eye was slightly wider than the DRA



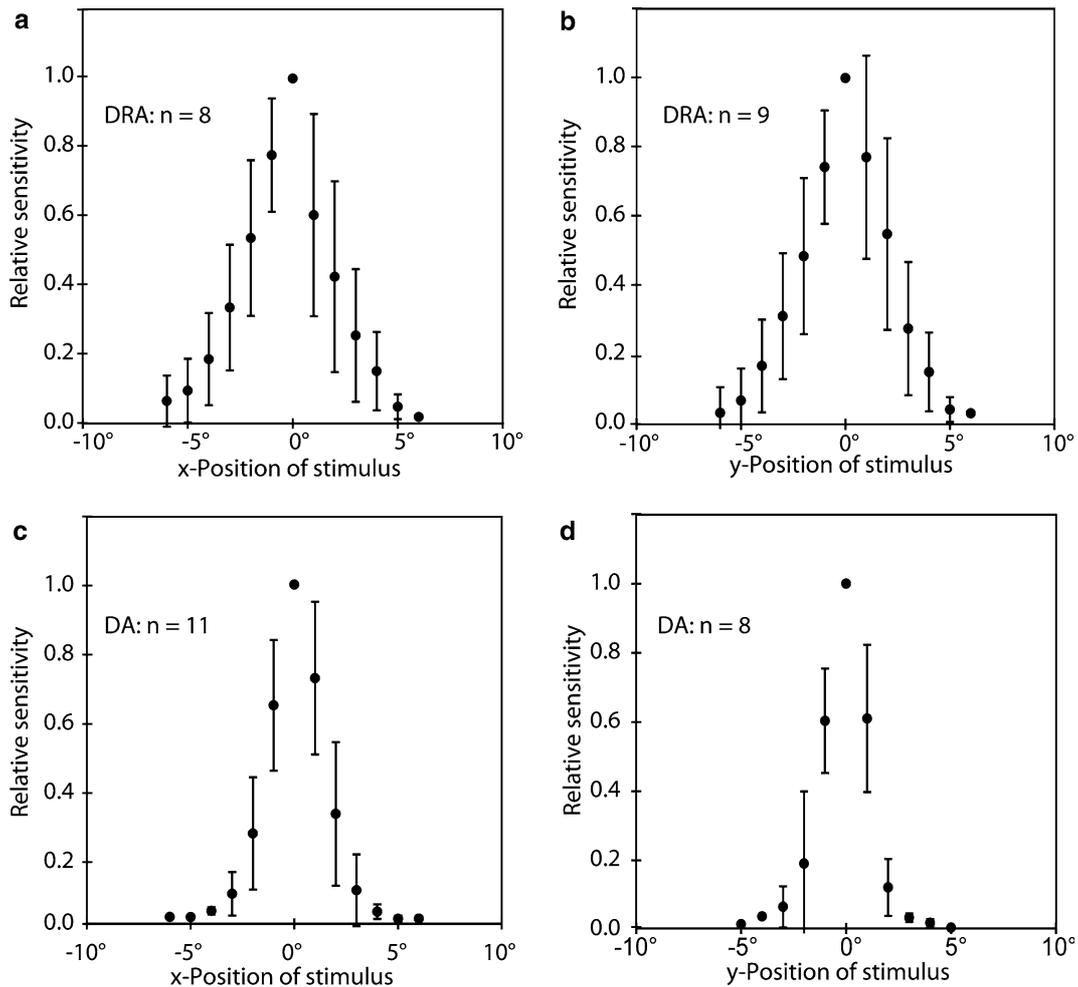
**Fig. 5** Optical axes of the various spectral types of photoreceptor recorded in the DRA and in the dorsal part of the main retina (DA) in space-related coordinates. The center of the diagram ( $90^\circ$ ) represents the long axis of the monarch eye which was assumed to be oriented vertically during free flight (as seen from lateral). Cells were recorded in the right eye; the grey shaded area therefore represents the ipsilateral visual field and the unshaded area the contralateral visual field. Green (empty circles), blue (empty squares) and UV receptors with a secondary green maximum (empty triangles) were recorded in the DA, whereas UV cells without a notable secondary maximum (filled triangles) belong to the DRA. Optical axes from DA receptors and DRA receptors are well separated along a line defining approximately  $10^\circ$  contralateral view (dashed line)



**Fig. 6** Histograms of polarization sensitivity (PS) values. **a** UV receptors of the DRA; ( $\overline{PS}=9.4 \pm 4.1$  [ $\pm$  SD],  $n=29$ ). **b** UV receptors of the DA ( $\overline{PS}=2.9 \pm 1.4$ ,  $n=4$ ). **c** Green receptors of the DA ( $\overline{PS}=2.8 \pm 0.9$ ,  $n=22$ ). The PS of DRA receptors is significantly higher than that of UV or green receptors in the DA ( $P < 0.001$ ; Mann–Whitney tests on ranks).  $N$  number of occurrences (cells).

so that ommatidia of the adjacent DA were also stimulated (see [Methods](#)). However, in a few cases we were able to restrict the paint screen in such a way that only DRA facets were exposed. In these cases, the ERG response was virtually absent in the UV and the spectral sensitivity curves closely matched those of single UV receptors in the DRA.

In all insects investigated so far, including monarchs, the polarization vision system is monochromatic, i.e., the polarization sensors in the DRA are all of the same spectral type (homochromatic). This makes the polarization vision system insensitive to changes in the spectral composition of a stimulus (Labhart and Meyer 1999). Each ommatidium in the DRA contains two sets of homochromatic polarization sensors that are sensitive to orthogonal e-vector orientations. This arrangement allows for polarization antagonism that, in effect, enhances PS and makes the system insensitive to fluctuations in ambient light intensity (for reviews, see Labhart and Meyer 1999; Wehner and Labhart 2005, in press). The number of polarization sensors per ommatidium varies between insects: there are two in flies, three in honey bees, and six in desert ants (Labhart and Meyer 1999). The monarch butterfly is the first insect in which both the opsin expression pattern and electrophysiological data suggest that all eight photoreceptors are monochromatic and involved in polarized light perception. Our findings indicate that monarch butterflies, like honey bees (von Helversen and Edrich 1974; Labhart 1980), desert ants (Duelli and Wehner 1973; Labhart 1986) and flies (von Philipsborn and Labhart 1990; Hardie 1984), exploit the UV range of skylight for e-vector navigation. In contrast, cricket polarization vision is mediated by blue receptors (Labhart et al. 1984; Herzmann and Labhart 1989) and the DRA of cockchafers is dominated by green receptors (Labhart et al. 1992). Why do insects exploit skylight polarization in different spectral ranges? Although this question has been studied using theoretical approaches (Seliger et al. 1994; Bartha and Horvath 2004), in our opinion the problem remains unresolved. On the one hand, skylight is polarized at all wavelengths. Thus, the choice of the spectral receptor type for polarization sensors may actually be of little relevance. Furthermore, the development of specialized photoreceptors for detecting skylight polarization in different insect taxa seems to have evolved independently (Labhart and Meyer 1999) and, thus, the spectral range used for polarization vision may be a taxonomic trait. On the other hand, both the degree of polarization and the spectral composition of skylight depend on meteorological conditions and time of the day (e.g., clear vs. cloudy, daylight vs. twilight) (Coulson 1988; Coemans et al. 1994; Labhart 1999). Thus, polarization vision may have been optimized for the prevailing sky conditions during the prevailing activity periods of different insects (Bartha and Horvath 2004; Zufall et al. 1989). Lepidopterans would be suitable to test these suggestions since they include strictly diurnal species, such as the monarch butterfly as well as nocturnal members, such as the moth



**Fig. 7** Average angular sensitivity functions of photoreceptors in the DRA and in the DA of the eye. In both the DRA (**a**, **b**) and the DA (**c**, **d**), angular sensitivities in  $x$ - and  $y$ -direction did not differ ( $P \geq 0.49$ , Mann–Whitney test on ranks) and therefore  $x$ - and  $y$ -data were pooled. The average acceptance angle (half-width at 50%

sensitivity) in the DRA was  $4.0^\circ \pm 1.1^\circ$  ( $\pm$ SD;  $n = 17$ ), in the DA it was  $2.6^\circ \pm 0.5^\circ$  ( $n = 19$ ). Thus, the visual fields of ommatidia in the DRA are somewhat wider than in the DA ( $P < 0.001$ , Mann–Whitney test on ranks).  $n$  number of cells tested.

*Deilephila elpenor*, which also exhibits an anatomically specialized DRA (Meincke 1981).

#### Polarization sensitivity

The DRA photoreceptors of monarch butterflies exhibit a high polarization sensitivity ( $\overline{PS} = 9.4$ ), similar to that found in other insects such as crickets (Labhart et al. 1984; Blum and Labhart 2000), honey bees (Labhart 1980), desert ants (Labhart 1986) and dung beetles (Dacke et al. 2002). The high PS is consistent with anatomical specializations observed in the monarch DRA: the microvilli of the rhabdoms are straight and well aligned along the rhabdom, which is shorter and wider than in the main retina (Labhart and Baumann 2003; Reppert et al. 2004; T. Labhart unpublished). These anatomical features along with the presence of two orthogonal microvilli orientations in each rhabdom have been referred to as ‘hallmarks of

polarized skylight detection’ (Labhart and Meyer 1999). Although the mean PS of DRA receptors is high, the recorded PS of single cells varies widely, ranging from 2 to 16.6. Such a strong scatter of PS has also been observed in the DRA of bees and crickets (Labhart 1980; Blum and Labhart 2000). Using a combination of intracellular recordings and dye marking, Blum and Labhart (2000) demonstrated that artificial electric coupling between receptors tuned to different e-vector directions significantly lowers the recorded PS. In crickets, the median PS value of all recorded photoreceptors in the DRA was 6.2, but after sorting out the recordings from coupled cells the value increased to 9.8. The same effect may account for the wide distribution of individual PS values recorded in the monarch DRA (Fig. 6a). Thus, the average PS of 9.4 probably underestimates the true PS of the monarch polarized light detectors.

In the main retina, UV and green receptors both exhibit low polarization sensitivities, which is in agree-

ment with anatomical findings: In the rhabdom cross-sections of butterfly eyes the arrangement of the microvilli appears somewhat irregular (Kolb 1985; Labhart and Baumann 2003) and the microvilli of individual receptors are not well aligned along the rhabdom (Kolb 1985). Such a microvillar misalignment significantly reduces PS (Nilsson et al. 1987). In the main retina, PS is kept low to avoid significant interference with the color vision system. Polarized reflections from glossy plant surfaces can produce false colors if the three spectral types of receptors are strongly polarization sensitive (Wehner and Bernard 1993). This may be the reason why in the strongly color dependent honey bee, PS of the photoreceptors in the main retina is reduced to  $< 2$  by a  $180^\circ$  twist of the rhabdom (Wehner et al 1975; Labhart 1980).

### Angular sensitivity

Monarch photoreceptors exhibit relatively narrow, bell-shaped angular sensitivity functions in both the DRA and the DA (Fig. 7). The mean visual field size of DRA receptors ( $4.0^\circ$ ) is only slightly wider than in the DA ( $2.6^\circ$ ). The electrophysiological findings in the monarch DRA are in agreement with a lack of optical specializations indicating strongly increased acceptance angles: upon visual inspection of the compound eye of a living monarch, the ommatidida of the DRA are undistinguishable from those in other parts of the eye, and histological sections indicate no obvious differences in the optical properties of DRA and DA ommatidia including the degree of pigmentation (T. Labhart, unpublished). In other insects, however, the visual fields of the ommatidia in the DRA are considerably enlarged. In these insects, the optics of the ommatidia in the DRA are specialized in ways that strongly increase the acceptance angles of the polarization-sensitive photoreceptors. For instance, the cornea can contain light-scattering structures (honey bees: Meyer and Labhart 1981; other hymenopterans: Aepli et al. 1985; cockchafers: Labhart et al. 1992), the screening pigment or the tracheal sheath, which normally shield the ommatidia from each other, can be missing or is reduced (crickets: Burghause 1979; locusts: Homberg and Paech 2002), or there can be a mismatch between focal length and rhabdom position (cockchafers: Labhart et al. 1992; crickets: Ukhanov et al. 1996). Increased visual fields in the DRA have been interpreted as an adaptation to the perception of polarized light under partly cloudy conditions. The integration over a large area of the sky reduces the effect of irregularities within the pattern of polarized skylight caused by clouds (Labhart 1999). However, enlarged visual fields do not seem to be essential for polarized skylight navigation: In the desert ant *Cataglyphis*, which uses the pattern of polarized skylight as a key compass cue during extended foraging trips (see e.g. Wehner 1994, 1997), the visual field size of

the DRA receptors ( $5.4^\circ$ , Labhart 1986) falls within the same order of magnitude as that found in the monarch.

### DRA-mediated polarization vision and behavior

Our study shows that the photoreceptors of the monarch DRA are highly polarization sensitive and suggests that monarchs use the UV part of the skylight spectrum for polarization vision. However, further investigations are needed to understand in which contexts monarchs make use of their polarized light detectors. Monarch butterflies use a time-compensated sun compass to orient during their annual autumn migration towards the overwintering sites in Central Mexico (Perez et al. 1997; Mouritsen and Frost 2002; Froy et al. 2003; Stalleicken et al. 2005). Previous behavioral studies have therefore focused on the role of polarized light vision for migratory orientation. Whether or not monarchs use the pattern of polarized skylight for compass orientation as part of the celestial compass system during migration is still under discussion (Reppert et al. 2004; Saumann et al. 2005; Stalleicken et al. 2005). Since monarchs are capable of time-compensated sun compass orientation with their DRA painted over (Stalleicken et al. 2005), it is at least clear that polarized light input is not necessary for migratory orientation in the monarch. Representatives of all six Lepidopteran families (Sphingidae, Satyridae, Saturniidae, Tortricidae, Noctuidae and Nymphalidae) examined so far show an anatomical specialized DRA (Meinecke 1981; Kolb 1985, 1986; Anton-Erxleben and Langer 1988; Hämmerle and Kolb 1996; White et al. 2003). These species, migrants and non-migrants, differ considerably in their ecology, habitat and life history, suggesting that polarized light detection is a general feature of Lepidoptera, for instance used during foraging. Systematic behavioral studies on the use of polarized light in lepidopterans are necessary.

**Acknowledgements** The Volkswagen Stiftung (Nachwuchsgruppe grant to H. M.), University of Oldenburg (to H. M. and J. S.), the DAAD (to J. S.) and the Swiss National Science Foundation grant 31-61844.00 (to T. L.) kindly provided financial support for this project. We would like to thank Gary D. Bernard for fruitful discussions.

### References

- Aepli F, Labhart T, Meyer E P (1985) Structural specializations of the cornea and retina at the dorsal rim of the compound eye in hymenopteran insects. *Cell Tissue Res* 239:19–24
- Anton-Erxleben F, Langer H (1988) Functional morphology of the ommatidia in the compound eye of the moth, *Antheraea polyphemus* (Insecta, Saturniidae). *Cell Tissue Res* 252:385–396
- Barta A, Horvath G (2004) Why is it advantageous for animals to detect celestial polarization in the ultraviolet? Skylight polarization under clouds and canopies is strongest in the UV. *J Theor Biol* 226:429–437

- Blum M, 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
- Briscoe AD, Bernard GD, Szeto AS, Nagy LM, White RH (2003) Not all butterfly eyes are created equal: Rhodopsin absorption spectra, molecular identification, and localization of ultraviolet-, blue-, and green-sensitive rhodopsin-encoding mRNAs in the retina of *Vanessa cardui*. *J Comp Neurol* 458:334–349
- Briscoe AD, Bernard GD (2005) Eyeshine and spectral tuning of long wavelength-sensitive rhodopsins: no evidence for red-sensitive photoreceptors among five Nymphalini butterfly species. *J Exp Biol* 208:687–696
- Burghause FMHR (1979) Structural specialization in the dorso-frontal region of the cricket compound eye (Orthoptera, Grylloidea). *Zool Jb Physiol* 83:502–525
- Burkhardt D, Streck P (1965) Das Sehfeld einzelner Sehzellen: Eine Richtigstellung. *Z Vergl Physiol* 51:151–152
- Coemans MAJM, Vos HJJ, Nuboer JFW (1994) The relation between celestial colour gradients and the position of the sun, with regard to the sun compass. *Vis Res* 34:1461–1470
- Coulson KL (1988) Polarization and intensity of light in the atmosphere. A Deepak Publishing, Hampton
- Duelli P, Wehner R (1973) The spectral sensitivity of polarized light orientation in *Cataglyphis bicolor* (Formicidae, Hymenoptera). *J Comp Physiol* 86:37–53
- Dacke M, Nordstrom P, Scholtz CH, Warrant EJ (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
- Eguchi E, Watanabe K, Hariyama T, Yamamoto K (1982) A comparison of electrophysiologically determined spectral responses in 35 species of Lepidoptera. *J Insect Physiol* 28:675–682
- Froy O, Gotter AL, Casselman AL, Reppert SM (2003) Illuminating the circadian clock in monarch butterfly migration. *Science* 300:1303–1305
- Hämmerle B, Kolb G (1996) Retinal ultrastructure of the dorsal eye region of *Pararge aegeria* (Linné) (Lepidoptera: Satyridae). *Int J Insect Morphol* 25:305–315
- Hardie RC (1984) Properties of photoreceptor-R7 and photoreceptor-R8 in dorsal marginal ommatidia in the compound eyes of *Musca* and *Calliphora*. *J Comp Physiol* 154:157–165
- Von Helversen O, Edrich W (1974) Der Polarisationsempfänger im Bienenaugen: ein Ultraviolettzeptor. *J Comp Physiol* 94:33–47
- Herzmann D, Labhart T (1989) Spectral sensitivity and absolute threshold of polarization vision in crickets: a behavioral study. *J Comp Physiol A* 165:315–319
- Homberg U, Paech A (2002) Ultrastructure and orientation of ommatidia in the dorsal rim area of the locust compound eye. *Arthropod Struct Dev* 30:271–280
- Homberg U (2004) In search of the sky compass in the insect brain. *Naturwissenschaften* 91:199–208
- Kolb G (1985) Ultrastructure and adaptation in the retina of *Aglais urticae* (Lepidoptera). *Zoomorphology* 105:90–98
- Kolb G (1986) Retinal ultrastructure in the dorsal rim and large dorsal area of the eye of *Aglais urticae* (Lepidoptera). *Zoomorphology* 106:244–246
- 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, Hodel B, Valenzuela I (1984) The physiology of the cricket's compound eye with particular reference to the anatomically specialized dorsal rim area. *J Comp Physiol* 155:289–296
- Labhart T (1986) The electrophysiology of photoreceptors in different eye regions of the desert ant, *Cataglyphis bicolor*. *J Comp Physiol A* 158:1–7
- Labhart T, Meyer EP, 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, Meyer EP (1999) Detectors for polarized skylight in insects: a survey of ommatidial specializations in the dorsal rim area of the compound eye. *Microscop Res Techn* 47:368–379
- Labhart T (1999) How polarization-sensitive interneurons of crickets see the polarization pattern of the sky: a field study with an optoelectronic model neurone. *J Exp Biol* 202:757–770
- Labhart T, Petzold J, Helbling H (2001) Spatial integration in polarization-sensitive interneurons of crickets: a survey of evidence, mechanisms and benefits. *J Exp Biol* 204:2423–2430
- Labhart T, Baumann F (2003). Evidence for a polarization compass in monarch butterflies. *Proc Neurobiol Conf Göttingen* 29:545
- Mappes M, Homberg U (2004) Behavioral analysis of polarization vision in tethered flying locusts. *J Comp Physiol A* 190:61–68
- Meinecke CC (1981) The fine-structure of the compound eye of the African armyworm moth, *Spodoptera exempta* Walk (Lepidoptera, Noctuidae). *Cell Tissue Res* 216:333–347
- Meyer E P, 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, Labhart T (1993) Morphological specializations of dorsal rim ommatidia in the compound eye of dragonflies and damselflies (Odonata). *Cell Tissue Res* 272:17–22
- Mouritsen H, Frost B J (2002) Virtual migration in tethered flying monarch butterflies reveals their orientation mechanisms. *Proc Natl Acad Sci USA* 99:10162–10166
- Nilsson D E, Labhart T, Meyer E P (1987) Photoreceptor design and optical properties affecting polarization sensitivity in ants and crickets. *J Comp Physiol A* 161:645–658
- Perez S M, Taylor O R, Jander R (1997) A sun compass in monarch butterflies. *Nature* 387:29
- Von Philipsborn A, Labhart T (1990) A behavioral study of polarization vision in the fly, *Musca domestica*. *J Comp Physiol A* 167:737–743
- Reppert SM, Zhu H, White RH (2004). Polarized light helps monarch butterflies navigate. *Curr Biol* 14:155–158
- Seliger HH, Lall AB, Biggley WH (1994) Blue through UV polarization sensitivities in insects: optimizations for the range of atmospheric polarization conditions. *J Comp Physiol A* 175:475–486
- Sauman I, Briscoe AD, Zhu HS, Shi DD, Froy O, Stalleicken J, Yuan Q, Casselman A, Reppert SM (2005) Connecting the navigational clock to sun compass input in monarch butterfly brain. *Neuron* 46:457–467
- Stalleicken J, Mukhida M, Labhart T, Wehner R, Frost B, Mouritsen H (2005). Do monarch butterflies use polarized skylight for migratory orientation? *J Exp Biol* 208:2399–2408
- Stavenga DG, Smits RP, Hoenders BJ (1993) Simple exponential functions describing the absorbency bands of visual pigment spectra. *Vis Res* 33:1011–1017
- Ukhanov KY, Leertouwer HL, Gribakin FG, Stavenga DG (1996) Dioptrics of the facet lenses in the dorsal rim area of the cricket *Gryllus bimaculatus*. *J Comp Physiol A* 179:545–552
- Wehner R, Bernard GD, Geiger E (1975) Twisted and non-twisted rhabdons and their significance for polarization detection in the bee. *J Comp Physiol* 104:225–245
- Wehner R (1982) Himmelsnavigation bei Insekten Neurophysiologie und Verhalten. *Neujahrsbl Naturforsch Ges Zürich* 184: 1–132
- Wehner R (1984) Astronavigation in insects. *Annu Rev Entomol* 29:277–298
- Wehner R, Bernard GD (1993) Photoreceptor twist: a solution to the false-color problem. *Proc Natl Acad Sci USA* 90:4132–4235
- Wehner R (1994) The polarization-vision project: championing organismic biology. In: Schildberger K, Elsner N (eds) Neural basis of behavioural adaption. G Fischer, Stuttgart, pp 103–143
- Wehner R (1996) Polarisationsmusteranalyse bei Insekten. *Nova Acta Leopoldina NF* 72:159–183
- Wehner R (1997) The ant's celestial compass system: spectral and polarization channels. In: Lehrer M (eds) Orientation and communication in Arthropods. Birkhäuser, Basel, pp 145–185

- Wehner R, Labhart T (2005) Polarization vision. In: Warrant E, Nilsson D-E (eds) Invertebrate vision. Cambridge University Press (in press)
- White RH, Xu HH, Munch TA, Bennett RR, Grable EA (2003) The retina of *Manduca sexta*: rhodopsin expression, the mosaic of green-, blue- and UV-sensitive photoreceptors, and regional specialization. *J Exp Biol* 206:3337–3348
- Wolf R, Gebhardt B, Gademann R, Heisenberg M (1980) Polarization sensitivity of course control in *Drosophila melanogaster*. *J Comp Physiol* 139:177–191
- Zufall F, Schmitt M, Menzel R (1989) Spectral and polarized-light sensitivity of photoreceptors in the compound eye of the cricket (*Gryllus bimaculatus*). *J Comp Physiol A* 164: 597–608