Spatial resolution and optical sensitivity in the compound eyes of two common wasps, *Vespula germanica* and *Vespula vulgaris.*

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1. ABSTRACT

Vespula germanica and *Vespula vulgaris* are two common European wasps that have ecological and economic importance due to their invasive introduction to many different countries and environments. Their success as organisms has been enabled partly by their vision, their visual behaviour and their capacity to learn visual cues in the context of homing and navigation. In general, diurnal hymenopterans are well known for their ability to memorise "visual snapshots" of landmarks along their foraging routes in order to orient themselves and to find their way back to the nest after foraging trips. However, the visual systems of *V. germanica* and *V. vulgaris* have not received any deep attention. We used electrophysiology, together with optical and anatomical techniques, to measure the spatial resolution and optical sensitivity of the compound eyes of both species. We found that both wasps have high anatomical spatial resolution with narrow interommatidial angles ($\Delta\varphi$ between 1.0 and 1.5°) and a distinct acute zone in the frontal-ventral part of the eye ($\Delta\rho$ below 1.3°). These narrow interommatidial angles are matched to photoreceptors having small angular sensitivities, indicating eyes of high spatial resolution well suited to their ecological needs. Additionally, we found that both species exhibit an optical sensitivity that is typical of other day-flying hymenopterans.

Key words: Electrophysiology, Omatidia, Optics, Vision, Visual Resolution, Wasps.

2. INTRODUCTION

The German wasp, *Vespula germanica,* and the Common wasp, *Vespula vulgaris*, are social wasps native to Europe that were accidentally introduced to the United States and Canada (MacDonald et al. 1980; Akre et al. 1989; Gambino 1991), Chile and Argentina (Edwards 1976; Masciocchi et al. 2010), South Africa (Whitehead and Prins 1975), New Zealand (Thomas 1960; Clapperton et al. 1989) and Australia (Spradbery 1973). Like most other insects, *V. germanica* and *V. vulgaris* have two types of visual organs, the ocelli and the compound eyes (Appendix, Figure A1). Many ocelli are capable of rapidly detecting changes in light intensity averaged over a wide visual field, and have a variety of purposes for insects (Mizunami 1994). In contrast, the compound eyes are sophisticated visual organs responsible for functions that require good spatial resolution, including motion detection, pattern recognition and colour vision. Arthropods possess two major types of compound eyes (Land 1981): apposition compound eyes, which are typical of day-active insects that fly in bright light, and superposition compound eyes, a highly sensitive eye design typical of nocturnal insects. In apposition eyes,

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the ommatidia (visual units), are isolated from each other by a thick sleeve of dark light-absorbing screening pigment, meaning that light reaches the photoreceptors of each ommatidium exclusively through its small overlying facet lens (which represents the pupil of the apposition eye). Thus, for day-flying wasps the abundance of light allows the eye to maximize spatial resolution at the expense of sensitivity (Greiner et al. 2004).

V. germanica and *V. vulgaris* are considered predators and have a highly diverse diet (Harris 1991; Beggs et al. 2011; Archer and Penney 2012; Grangier and Lester 2012). Adults of these species exhibit a foraging behaviour feeding on carrion, garbage, live arthropods and fruits, and in larval stages they are fed honeydew by the foraging adults (Akre 1982). When exploiting a food source, foraging German wasps learn cues from the environment in order to retrieve memories related to rewarding stimuli (D'Adamo and Lozada 2011; Lozada and D'Adamo 20112011). Similar to other social species, foraging individuals of *V. germanica* collect food and return with it back to the nest. In social hymenopterans, finding and remembering food sources, as well as making several trips between the food source and the nest, is a frequent behaviour exhibited by foraging individuals. D'Adamo and Lozada (2007) have shown that *V. germanica* is able to learn visual cues while foraging, in order to find and memorize feeding sites. Foraging wasps use visual objects and local landmarks as beacons for signalling food location, and then as guides when attempting to relocate a food source (D'Adamo and Lozada 2007). Because they are able to return to a constant location, this suggests that individuals are able to perform complex and important cognitive tasks, such as learning and memorizing landmarks, thereby learning the locations associated with them (D'Adamo and Lozada 2007; 2011; Lozada and D'Adamo 2011).

In addition, *V. vulgaris* is capable of learning images of human faces (Warrington 1996) and is able to discriminate between a face stimulus learned during training from completely novel faces that are presented together in a test situation (Avargues-Weber et al. 2017). This indicates that *V. vulgaris* is capable of performing complex visual tasks.

Diurnal hymenopterans are well known for their ability to recall a sequence of memorized "visual snapshots" of the landscape along their foraging routes in order to navigate between a nest and a foraging site (Collett et al. 2013). Ants, bees and wasps perform learning walks or flights that allow them to learn information about their environment and surroundings (Tinbergen 1938; Collett et al. 2016; Stürzl et al. 2016). It has been suggested that visual guidance in social hymenopterans relies on "storing" views of frequent routes, or of specific

locations (alignment image matching), allowing them to follow a familiar path (Collett et al. 2013; 2016).

Wasps are able to learn different aspects of visual cues, such as a landmark's colour, shape, position and distance, while performing these learning flights (Collett and Zeil 1996). Hence, it is likely that the ability of *V. germanica* and *V. vulgaris* to identify, learn, and memorize landmarks along the route and around the nest is limited by its visual system (e.g. the structure of its eyes) and by its cognitive abilities. However, while it has become clear that *V. germanica and V. vulgaris are* capable of quickly learning visual tasks in the context of food relocation (Moreyra et al. 2016), the cognitive abilities of the German wasp in relation to navigation have only received limited study (D'Adamo and Lozada 2007; 2011; Lozada and D'Adamo 2011; Moreyra et al. 2016; Avargués-Weber et al. 2017). Remarkably, the structure and physiology of their eyes has never been studied. To address this gap in our knowledge, we investigated the structures of the compound eyes of *V. germanica* and *V. vulgaris* as well as use electrophysiology to measure their visual spatial resolution. Besides this, we used histological and optical techniques (Greiner et al. 2004; Warrant et al. 2004) with recently developed methods for mapping physiological acuity and optical sampling across different regions of the eye (Rigosi et al. 2017, 2021). For the first time, the spatial visual resolution and visual acuity of these two species of wasps will be measure. We hypothesized that *V. germanica* and *V. vulgaris* have good spatial resolution and visual acuity that allows them to have good orientiation and navigate along the different environments.

3. METHODS

3.1 Animals

Individuals of *V. germanica* and *V. vulgaris* were collected between July and October 2021 at the campus of Lund University. All individuals were worker females and during experiments were kept overnight in a refrigerator at 8°C, and during the day in lab conditions (23–26°C) and were fed twice a week with two spoons of honey in a petri dish.

3.2 Electrophysiology

This procedure followed the protocol of Rigosi et al. (2017). Individuals of both species were captured and anaesthetised on ice (4°C) for around 20 minutes. In the meantime, the end of a pipette tube tip was cut off to make a narrow hole. The anaesthetised wasp was inserted into the tube and its head was allowed to emerge through the hole. To avoid any movement during recordings, the thorax, head, pedicel of the antennae and mouthparts were immobilised by waxing them to the pipette using a hot mixture of beeswax and violin rosin (1:1). Next, the wasp was mounted onto a holder and a triangular window (5–10 facets wide) was cut in the dorsal margin on the cornea of the left eye in order to insert an electrode and permit intracellular recordings within the wasp's left lateral field of view (Fig. 1A). In some cases, the wasp was flipped 90° and a hole of the same size was made near the ventral margin of the right compound eye, allowing recordings from the fronto-ventral part of the eye. After the hole was cut, it was covered with Vaseline to prevent the inner ocular tissues from drying out. A reference electrode of thin silver chloride coated silver wire was inserted into the other eye. Intracellular membrane potentials of single photoreceptors were recorded using an amplifier with a low noise, high-input impedance headstage (NPI BA-03X). Sharp electrodes were fabricated from aluminosilicate glass capillaries (SM100F-10, Harvard Apparatus) pulled on a Sutter Instruments P-87 puller and filled with 1 M KCl solution. Electrode resistance was 80–240 MΩ. A piezo-controlled manipulator allowed the electrode to be stepped through the retina in steps of 3–7 μm. Successful penetration of photoreceptors was revealed by large amplitude modulation of the membrane potential in response to full screen flicker stimuli, with depolarization to brightening events (Fig. 1B). As many repetitions as possible were made for every cell. In total, 50 photoreceptors were used in the analysis for both species, 24 for *V. germanica* and 26 for *V. vulgaris*.

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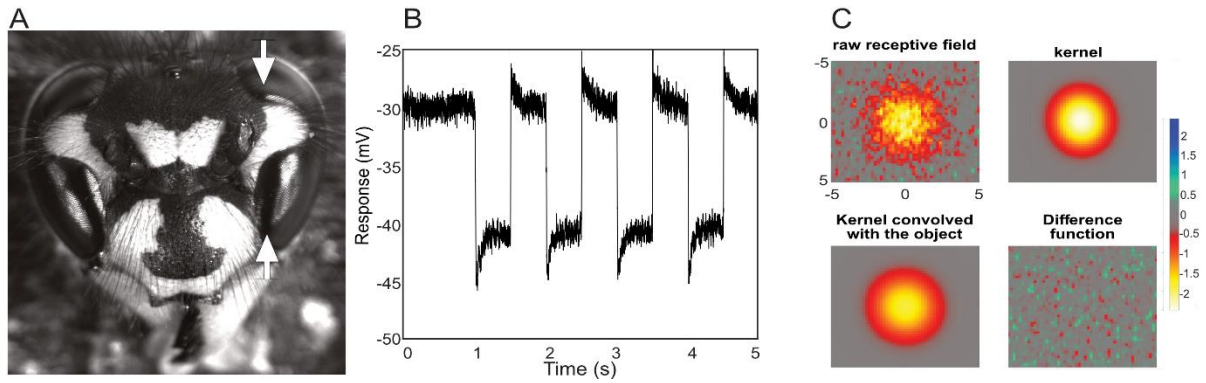
Figure 1. A. Location where the electrode was introduced in order to obtain frontal and lateral photoreceptor recordings (*arrow* in *V.vulgaris*). **B**. Photoreceptor responses to a flickering stimulus of the whole screen. **C**. Electrophysiological results taken from a lateral photoreceptor in *V.vulgaris*, showing the raw data of the receptive field of the photoreceptor recorded (5° x 5°). A 2D Gaussian kernel was then fitted to this data to take account of the finite size of the object used to measure it (i.e. an 2.0° by 2.0° black square target in this case). A model fit was obtained by 2D convolution between the estimated kernel and the stimulus at the same spatial scale. Model parameters were iteratively fitted to reduce the error (difference function) between the convolved model and the raw receptive field.

3.3 Receptive field (RF) scans and angular sensitivity ($\Delta\rho$) estimation of single phtoreceptors

One black bar (5°× 144°, velocity: 96°/s) was presented on the stimulus screen (Asus VG279QM, resolution 1920x1080 at 280 Hz, using custom-written software in Matlab) and was moved across the screen along the four cardinal directions, allowing us to estimate the centre-location of the receptive field of each photoreceptor. In response to the movement of a black bar across its RF, the photoreceptor experienced a dimming that resulted in a hyperpolarisation of its membrane potential. These responses allowed us to have an estimate of the centre coordinates of the photoreceptors RF on the screen and to generate a stimulus sequence around its centre to accurately measure the receptive field of the photoreceptor. A region of interest (ROI, 9° square) was centred on the centre of the receptive field (RF) and a small black square (Weber contrast = −0.998) drifted left to right (velocity 48°/s), and from top to bottom, within the ROI to generate 61 sequential raster lines (i.e. with a vertical scanning resolution of 0.15°). With a pre-stimulus recording time of 0.5 s the total scanning time required to obtain a complete RF was approximately 120 s. The data obtained fed a model that allowed us to fit the measured receptive fields by convolving a 2D Gaussian kernel (Fig. 1C) with an image of the size and shape of the target rendered at the same resolution as the raster plot. For frontal recordings the stimulus target was 1.5°× 1.5° or 2.0° × 2.0°, while for recordings from the lateral field of view the target was 3.0° × 3.0°. The summed squares of the difference (Fig. 1C lower right) between the convolved image and the observed data was then minimised using a simplex search that varied the kernel dimensions. Horizontal and vertical photoreceptor acceptance angles ($\Delta\rho$, in

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degrees) were then estimated from the best-fit kernel. The same model also allowed us to identify the exact centre coordinates of the RF for subsequent experiments. In fitting a 2D Gaussian (linear) kernel (O'Carroll and Wiederman 2014) we assume linearity in the measured signal, despite the potential recruitment of voltage-gated conductances. We therefore selected a target size for the black object that was small enough to limit maximum responses to below 5.5 mV, which we previously showed maintains reasonable linearity in photoreceptor recordings from honeybee foragers (Rigosi et al. 2017).

3.4 Receptive field location

For each photoreceptor recorded, the coordinates of the receptive field location on the eye were measured. The distance from the central midpoint in the frontal eye (0° azimuth (longitude), 0° elevation (latitude)), and perpendicular to the dorsal head axis, was measured in pixels and transformed to degrees (by using a degree/pixel ratio calculated for each cell).

3.5 Analysis

All analyses were made in Matlab 2015b. For each wasp, the visual angle subtended by each pixel (α) was calculated as follows:

$$\alpha = 2 \arctan[(a/a1)/2D], \qquad [1]$$

where *a* is the screen width in centimetres, *a1* the screen width in numbers of pixels, and *D* is the distance from the screen in centimetres.

Figure 2. Thoracic injection site for Lucifer Yellow, at 1 mm depth, in *V. germanica.*

3.6 Spatial resolution: Optical measurements of interommatidial angle, $\varDelta\varphi$

Female wasps (N=1) were anaesthetised using CO_2 and then mounted on custom-made 3D printed holders (Appendix, Figure A2A). While the individual was sedated, it was immobilized by waxing the abdomen, wings, thorax, mouthparts and antennae with the same hot wax mixture used for electrophysiology (beeswax and violin rosin 1:1). The method described by Rigosi et al. (2021) was followed with slight modifications. Rather than applying a fluorophore crystal directly within the head, four microliters of Lucifer Yellow (6% concentration in distilled water) was injected (using a 100µl Hamilton syringe fitted to a KD Scientific injector model: LEGATO111) at 1 mm depth in the thorax, as a means to reach the aorta with the fluorescence dye, at a flow rate of 1 uL/minute (Fig. 2). The injection of fluorescent Lucifer Yellow allowed the visualisation of the luminous pseudopupil, the small region of ommatidia on the eye surface directed towards the viewer. Once the injection was complete, animals were mounted in a custom-built goniometer constructed from two motorized precision rotation stages (KPRMTE/M, Thorlabs Inc., USA) on a manual translation stage (all components from Thorlabs Inc., USA), using a custom-made 3D printed holder that allowed the subject to rotate around its own axis (Appendix, Figure A2B).

The holder, together with the goniometer, allowed continuous or stepwise rotation of the animal across both azimuth (longitude) and elevation (latitude), allowing us to manipulate the three goniometer axes: dorsal-ventral (yaw), anterior-posterior (roll), and left-right (pitch) of the wasp's head. We were able to obtain pictures at up to 90° latitude and 100° longitude. After the animal was placed on the goniometer, Lucifer Yellow dust was sprinkled on the wasp's eye to create landmarks over the eye surface (used to identify the same ommatidia in sequential photographs explained below).

At each 10° step of latitude and longitude, an image of the eye surface, including the luminous pseudopupil and landmarks, was captured using a Nikon SMZ18 fluorescence stereomicroscope (Nikon, BergmanLabora AB, Sweden). This microscope had been modified by a 180° reversal of the imaging head and a rotation of the objective turret to align the episcopic light source (Sola light engine SM-5-LCR-SB Lumencor®, USA) coaxially with the imaging pathway of a cooled sCMOS camera (Andor Zyla 5.5, Oxford Instruments) coupled to NIS Elements AR software (version 4.50, Nikon, BergmanLabora AB, Sweden).

Using these photographs, we were able to determine the facet coordinates of the facet located at the centre of the luminous pseudopupil in each photograph (i.e. at each latitude and longitude in the 10° grid across the eye). These facet coordinates were determined after

defining X and Y facet rows relative to an "origin facet" located at a latitude and longitude of 0° (see Warrant et al. (2004) for a full description of the methods). Using custom-built software (Facet 4.0), these coordinates were used to calculate the ommatidial density at each location in the eye (in ommatidia per square degree), and thus the local average interommatidial angle $\Delta\varphi$ (degrees). These values of $\Delta\varphi$ were plotted on a sphere representing three-dimensional space around the wasp and contour lines were interpolated to connect regions having the same $\Delta\varphi$.

3.7 Histology

Wasps were immobilized by keeping them at 8°C in a refrigerator for 40 minutes. Following this, they were inserted within a pipette tip whose end was cut to make a hole narrow enough for only the wasp's head to pass through. The wasp's head was then shaved using the rear edge of a razor blade, and subsequently the head was removed. From there, the mouthparts, antennae and cuticle were removed to keep only the eye, that was later transferred into fixative (a mixture of 2.5% glutaraldehyde and 2% paraformaldehyde in phosphate buffer, pH 7.2–7.5). The heads were fixed for 2–3 h at 4°C before being osmicated (2% OsO_4 in distilled H_2O) for 1 h. The heads were subsequently dehydrated in an ethanol series, transferred to propylene oxide and embedded in epoxy resin (Fluka). Frontal, longitudinal and tangential serial sections, 3 μm thick, were cut on a Reichert Ultracut microtome using glass knives. The 3-μm-thick section series were placed on microscope slides and flattened on a 60°C hot-plate, after which they were stained with Mallory's borax-methylene blue. and viewed under a Zeiss photo-microscope. Colour pictures were taken with Zeiss Axiophot microscope with a Nikon DS-Fi1c camera and NIS-Element D4.20.01 software. After the dissections were made, we calculate the optical sensitivity of the eye in white light illumination, S (in units of $\mu m^2 sr$: Land 1981; Warrant and Nilsson 1998):

$$S = \left(\frac{\pi}{4}\right)^2 D^2 \left(\frac{d}{f}\right)^2 \left(\frac{kl}{2.3+kl}\right), \qquad [2]$$

where D corresponds to the facet diameter, f the focal length of the eye, d and l corresponds to the diameter and length of the photoreceptor (rhabdom), and k is the absorption coefficient of the photoreceptor (taken as 0.0067 μm^{-1}: Bruno et al. 1977). This equation allows us to describe the light capturing capacity of the eye of the wasps in comparison to other species of insects.

3.8 Image analysis

ImageJ was used to measure the dimensions of visual structures. For both species (N=2 for each species) five (n=5) rhabdoms were measured for the diameter (f), while for the length of the ommatidia (L), n=5 for *V. germanica* and n=7 for *V. vulgaris*. For each species, each rhabdom was measured and the stadanrd deviation (SD) was calculated.

4. RESULTS

4.1 General description of the eye

Both *V. germanica* and *V. vulgaris* have well-formed compound eyes and three ocelli on the dorsal surface of the head (Fig. 3). They also both possess a cuticular "peninsula" that partially divides the eye into a dorsal and ventral part (Fig. 3). It was surmised that this cuticular indentation might create a "blind spot" in the frontal visual field, however as we will see below, this was not the case.
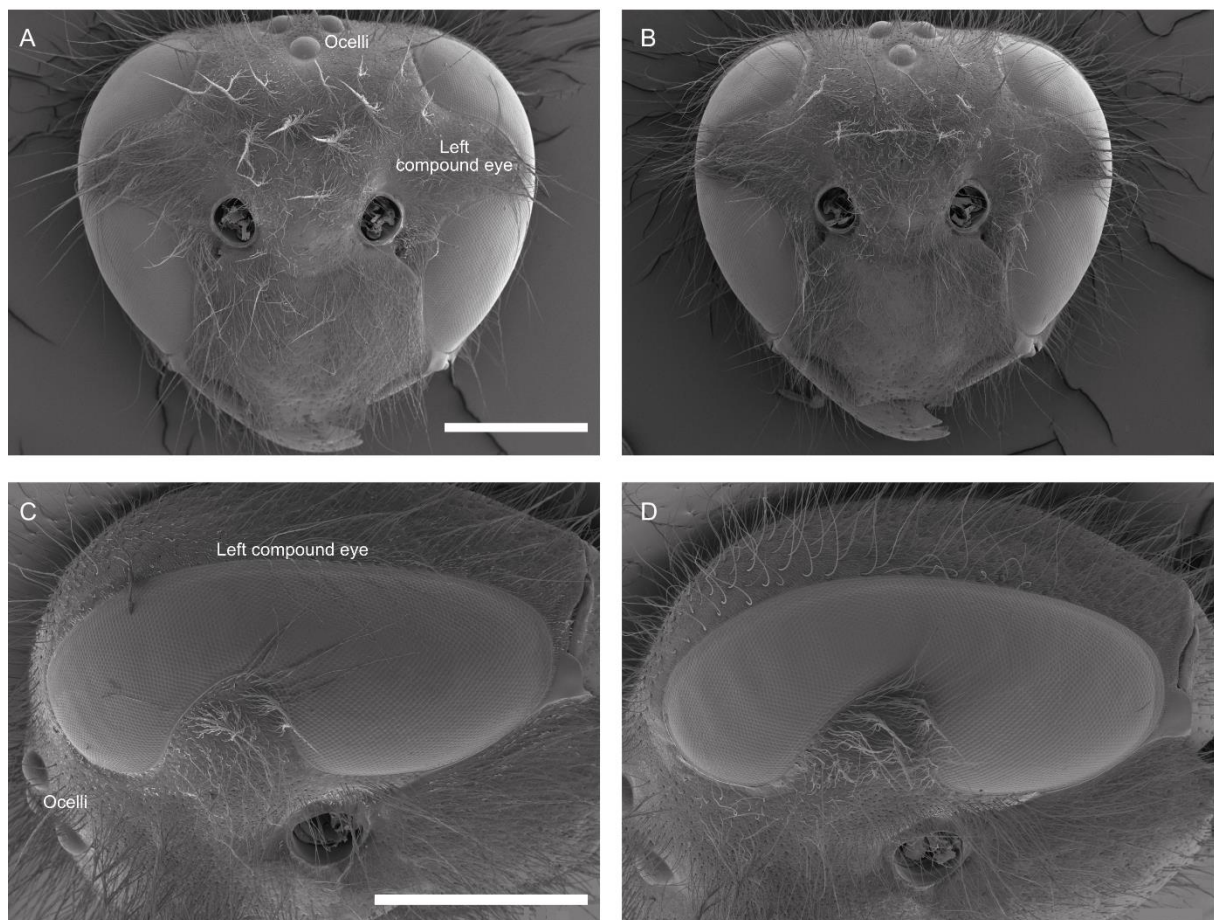
Figure 3. Scanning electron microscope images of dorso-frontal views of the heads, and closer lateral images of the eyes, of *V. germanica* (**A**, **C**) and *V. vulgaris* (**B**, **D**). Both scale bars: 1 mm (scale in A applies to B, and scale in C applies to D).
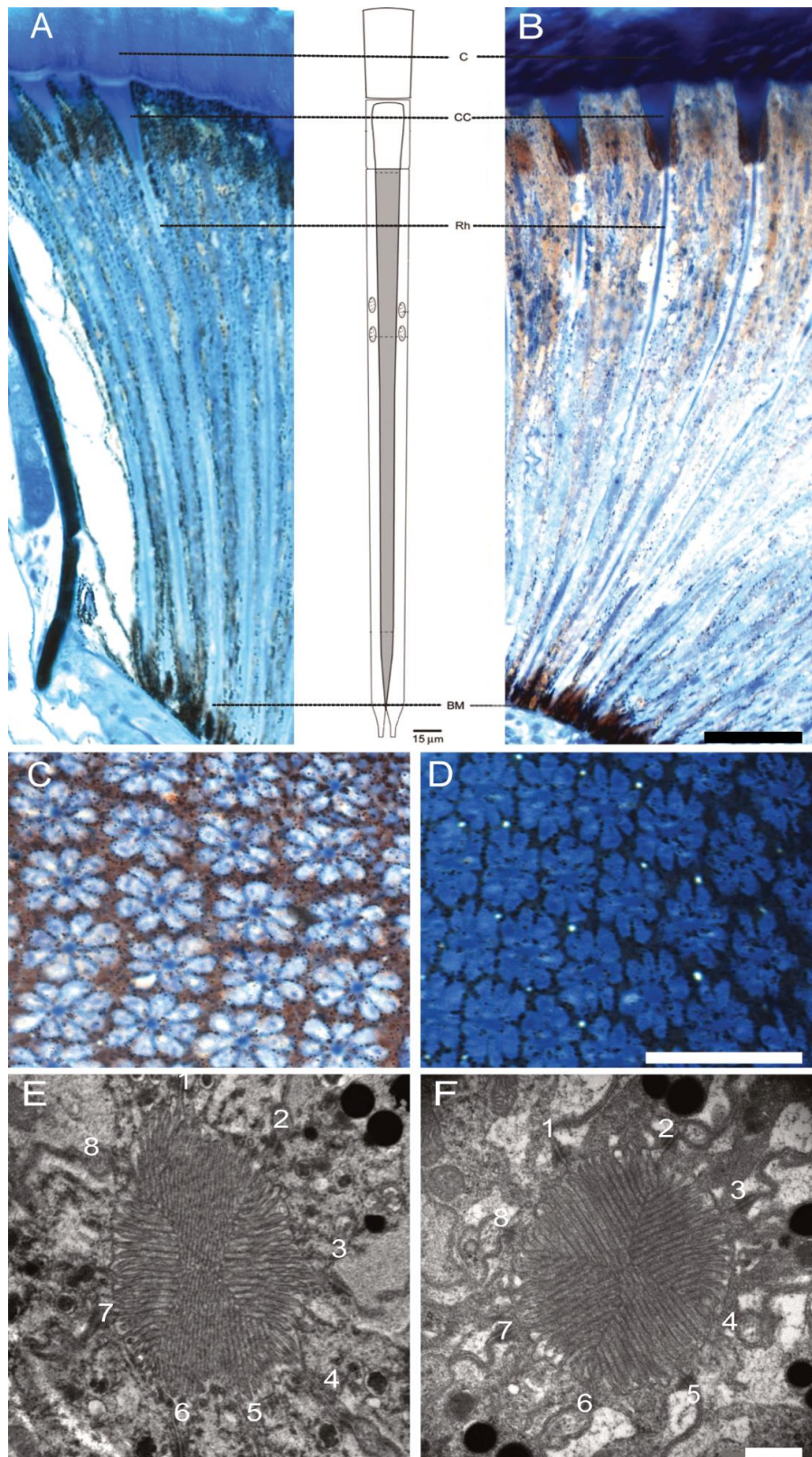
Figure. 4. **A, B**. Longitudinal sections through the apposition compound eyes of female *V. germanica* (A) and *V. vulgaris* (B) with a semi-schematic drawing of an ommatidium (centre, adapted from Greiner et al. 2004). Corneal facet (C), crystalline cone (CC), fused rhabdom (Rh) and basement membrane (BM). Scale bar: 50 μm (scale in B applies to A). **C, D**. Transverse sections of ommatidia in female *V. germanica* (C) and *V. vulgaris* (D). Scale bar: 50 μm (scale in D applies to C). **E, F**. Transmission electron micrographs of cross-sections through the rhabdoms of *V. germanica* (E) and *V. vulgaris* (F). In both species the retinula cells are visible and numbered. Scale bar: 500 μm (scale in F applies to E).

4.2 Internal eye morphology and optical sensitivity

The eyes of both wasp species are classical afocal apposition eyes (Fig. 4). The length of the rhabdom (l) in *V. germanica* was 227 ± 2.1 µm, and in *V. vulgaris* 255 ± 1.5 µm (Fig. 4A,B). We found that the pseudopupils of these species become divided by the cuticular peninsula, with both the dorsal and ventral eye parts revealing a portion of the pseudopupil simultaneously (at the upper and lower edges of the peninsula, respectively (Fig 5). This implies that rhabdoms on both the dorsal and ventral sides of this pensinsula view adjacent points in space, so that there is no blind spot caused by the cuticular peninsula.

Both species have the largest facet diameter just ventral to the cuticular peninsula, in the fronto-ventral zone of the eye (Fig. 6). From there, the facet diameter decreases smoothly towards the ventral region of the eye. Likewise in the dorsal part of the eye, facet diameter decreases smoothly frontally to dorsally. Although both species are very similar, *V. germanica* has the largest facet diameters (29 µm) while the largest facet diameters found in *V. vulgaris* were around 26 µm (Fig. 6).

Transmission electron micrographs of transverse sections through the rhabdoms from this frontal eye region reveal the diameter of the rhabdoms (d) (Fig. 4E,F) to be 2.1 ± 3.1 µm in *V. germanica* and 1.9 ± 2.7 µm in *V. vulgaris*. The focal length of the ommatidium (f) has previously been measured for *V. vulgaris* (Kelber et al. 2011) and was found to be 67 µm. We will use this value for both wasp species here.

With these parameters we were able to calculate the optical sensitivity S of the eyes in white light (Equation 2) at the centres of the acute zone for both species (where facet diameter D is 29 µm for *V. germanica* and 26 µm for *V. vulgaris* – Fig. 6): 0.20 µm²sr for *V. germanica* and 0.14 µm²sr for *V. vulgaris*.
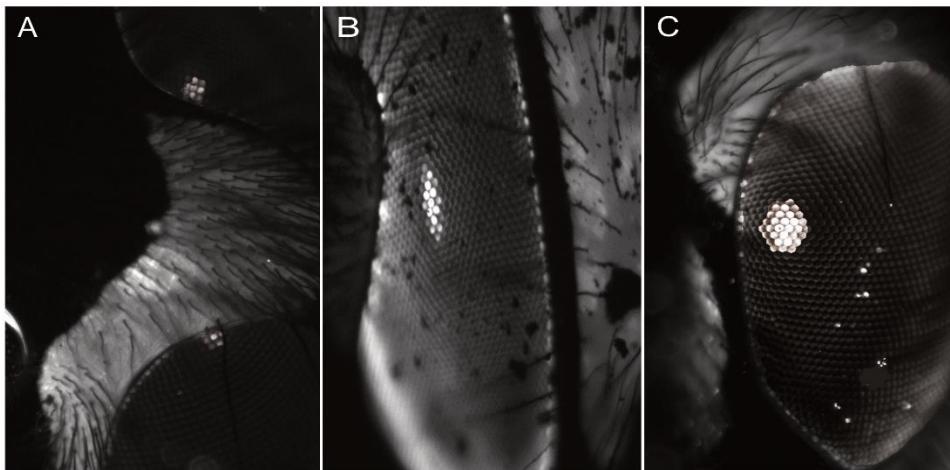

Figure 5. Luminous pseudopupil in *V. germanica*. (A) pseduopupil present in both dorsal and ventral "halves" of the eye. **(B)**, lateral side of the eye at 100° azimuth **(C)** and fronto-ventral zone where the acute zone was found.
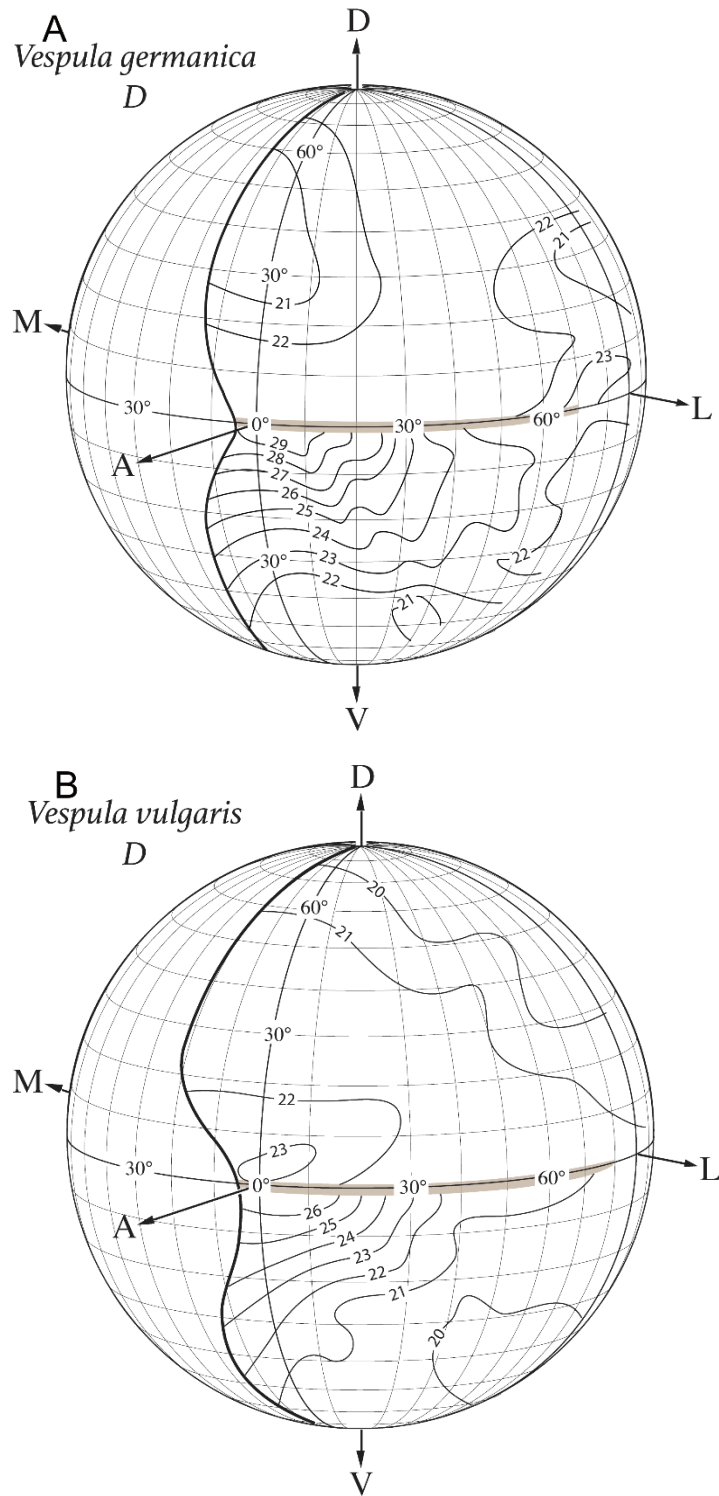
Figure 6: Facet diameters (*D*) in the left eye of a female *V. germanica* (**A**) and a female *V. vulgaris* (**B**). Data are plotted as isolines onto a sphere that represents the three-dimensional space around the wasp. Lines of latitude and longitude are shown in intervals of 10°. The boundary of the eye's visual field is also shown (*thick black line*). D = dorsal, *V* = ventral, *A* = anterior, M = medial and *L* = lateral. The *brown region* centred on latitude 0° represents the location of the "peninsula" of cuticle that penetrates the frontal eye but which does not create a "blind spot" within the visual field (it does however divide the eye into a dorsal and ventral half with differing spatial properties).
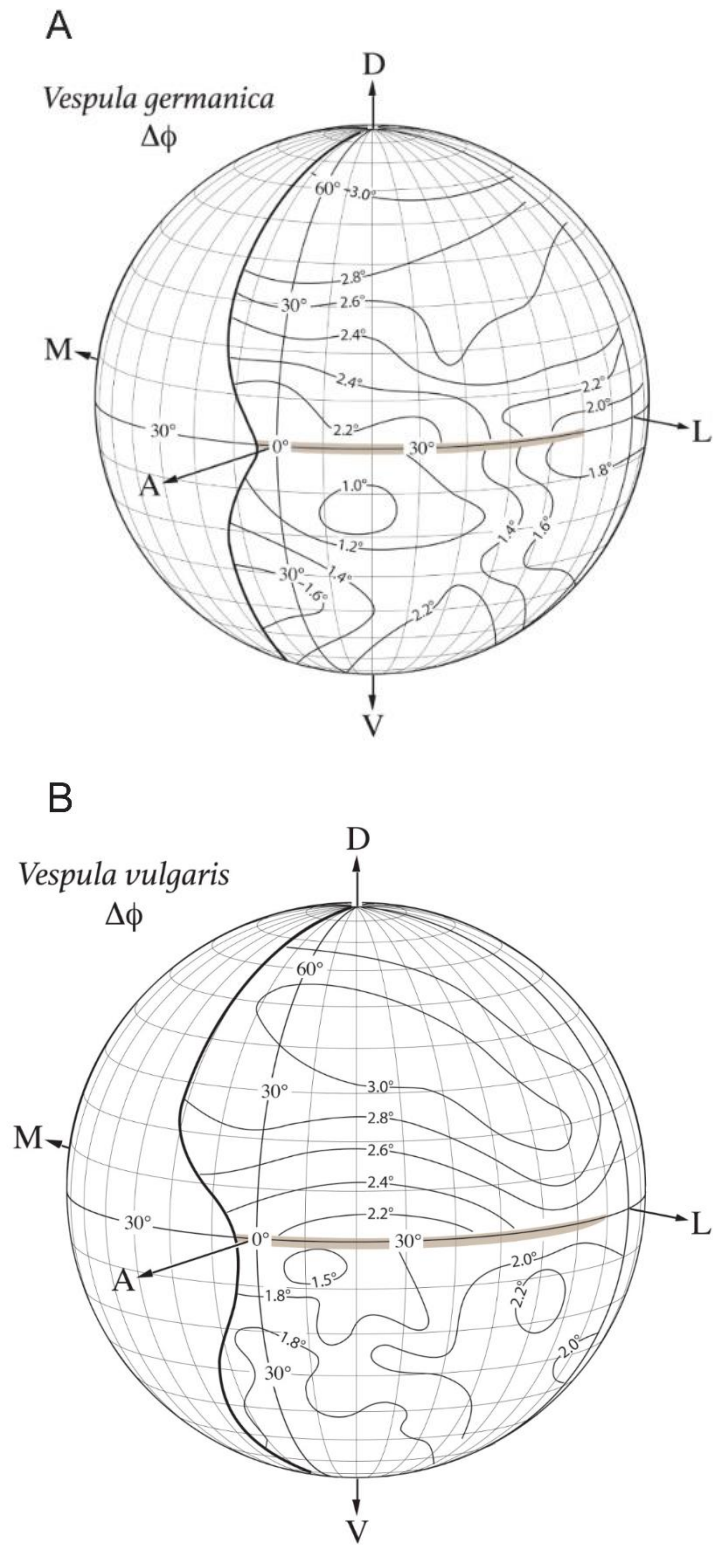
Figure. 7 Interommatidial angles ($\Delta\varphi$) in the left eye of a female *V. germanica* (**A**) and a female *V. vulgaris* (**B**). All other figure conventions as in Figure 5

4.3 Anatomical resolving power

In a compound eye, the packing density of ommatidia is represented by the angle between adjacent ommatidia. This is also known as the interommatidial angle $\Delta\varphi$, which determines the anatomical spatial resolution of the compound eye (Land 1981): the smaller $\Delta\varphi$, the greater the potential resolution. We found that the local $\Delta\varphi$ (averaged interommatidial angle) was the smallest in the frontal-ventral part of the eye in both wasps, around 1.0° for *V. germanica* between -10 and -20 degrees in latitude, and around 1.5° for *V. vulgaris* between 0 and -10 degrees in in latitude (Fig. 7). Thus, both wasps possess an "acute zone", a region of the eye (and thus visual field) having greatest spatial resolution. In both wasps the acute zone is positioned in the frontal eye, just below the horizontal equator. Beneath this acute zone, $\Delta\varphi$ increased smoothly towards the most ventral part of the eye. In contrast, the averaged interommatidial angle in the dorsal part of the eye (as delineated by the cuticular "peninsula" dividing the eye), behaved differently compared to the ventral part of the eye (Fig. 7). The "peninsula" was aligned with the horizontal equator and we found that in both species, $\Delta\varphi$ is greater in the dorsal part of the eye than in the ventral part, i.e. there is a sharp discontinuity with lower resolution just above the visual horizon. The smallest values of $\Delta\varphi$ found in the dorsal eye were almost twice as large as the smallest found in the acute zone of the ventral eye (2.0° for *V. germanica* and 2.2° for *V. vulgaris* between 0 and +10 degrees of latitude for both species). From there, $\Delta\varphi$ increased smoothly towards the dorsal region of the eye, increasing to around 3.0° in both species.
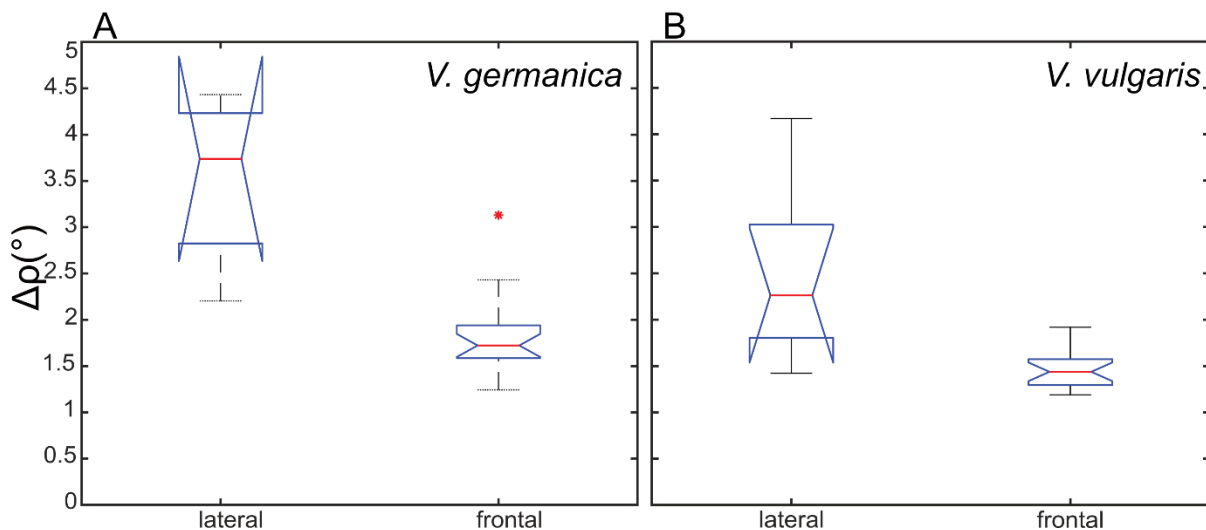
Figure 8. Acceptance angles ($\Delta\rho$ in degrees) for (**A**), *V. germanica* and (**B**) *V. vulgaris*. Outliers are shown in red.
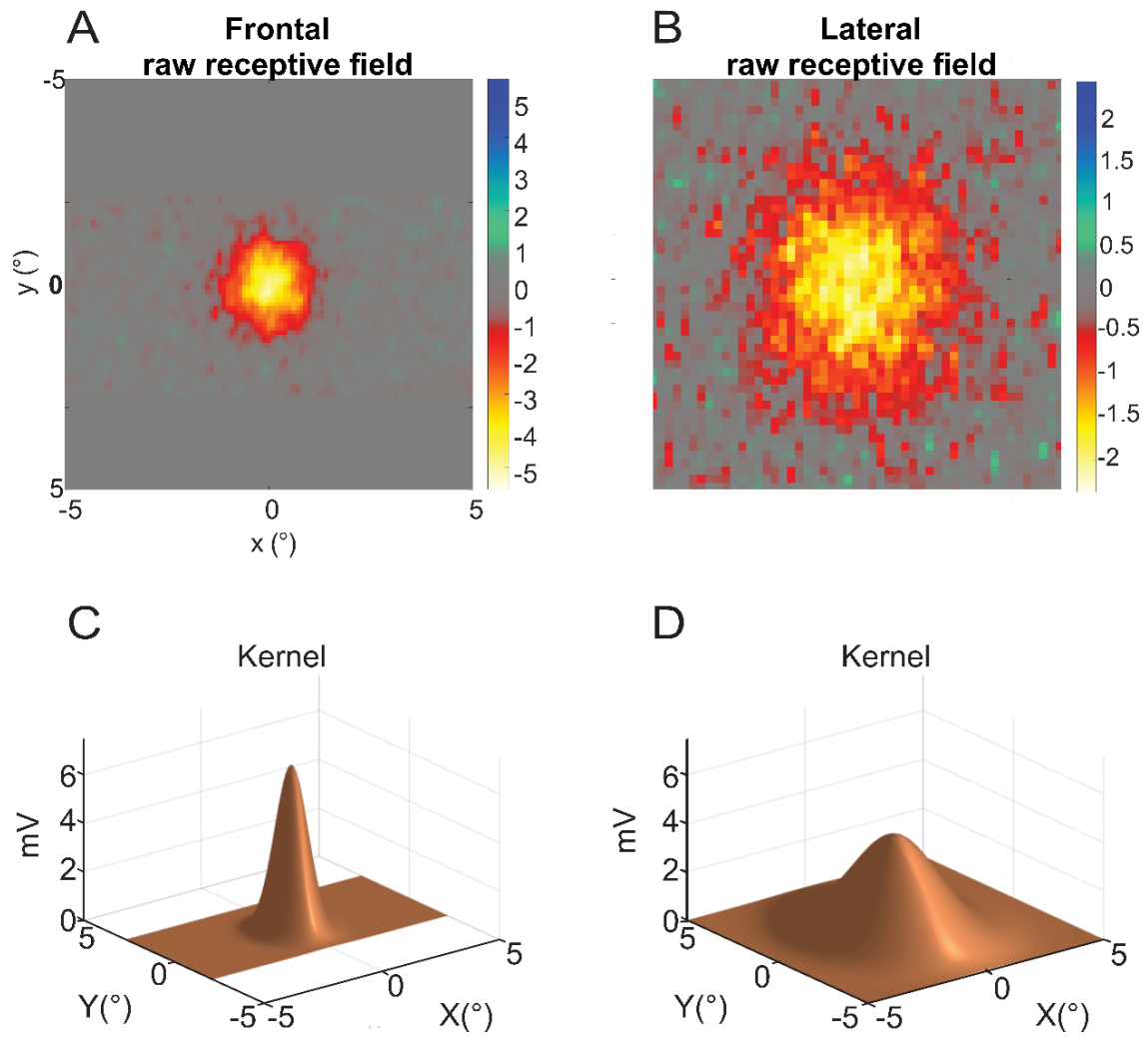
Figure 9. Comparison between data obtained from frontal (**A, C**) and lateral (**B, D**) photoreceptors. **A, B**. Raw data obtained from the photoreceptor where the size of the receptive field can be seen. X and Y are shown in degrees while the colour bar represents the different membrane voltages (mV). **C, D.** 2D Gaussian kernels that provide the best fit to the receptive field as described in Figure 1C

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4.4 Photoreceptor spatial receptive fields

Although we attempted to sample cells from a range of latitudes and longitudes, the overall variability in both recording location and receptive field width (i.e. acceptance angle $\Delta\rho$) was too large to allow us to directly generate a complete map of acuity at either the frontal or lateral location. Nevertheless, aggregating the data from the frontal and lateral regions (Fig. 7) confirms the higher acuity estimated optically for the frontal acute zone, with a median $\Delta\rho$ of 1.59° (Fig. 7A, averaged for both species, 95% confidence intervals 1.48° and 1.69°, n=39). The smallest values were located primarily around a region between 0 and 5° of longitude (with 9 of the smallest 10 values observed in this area) and between -10 and -20° of latitude (with 7 out of the 10 smallest values in this area), corresponding to the region of the eye with the smallest interommatidial angles (Fig 7). By comparison, the 11 cells recorded laterally revealed receptive fields almost twice as wide, with a median $\Delta\rho$ of 2.82° (Figs. 7,8)

Notably, we obtained several individual cells from the frontal eye region that could be held long enough to scan the receptive field several times, thereby obtaining high-quality receptive field maps, with $\Delta\rho$ below 1.3° degrees (Figs. 8, 9). A comparison of these values in both wasp species reveals that they are very similar (as shown by the plots in Figures 7B and 7C, and by the individual cell acceptance angle values plotted on the globe in Fig. 10): the sharpest values of $\Delta\rho$ are found frontally and the coarser values are found laterally (Fig. 9).

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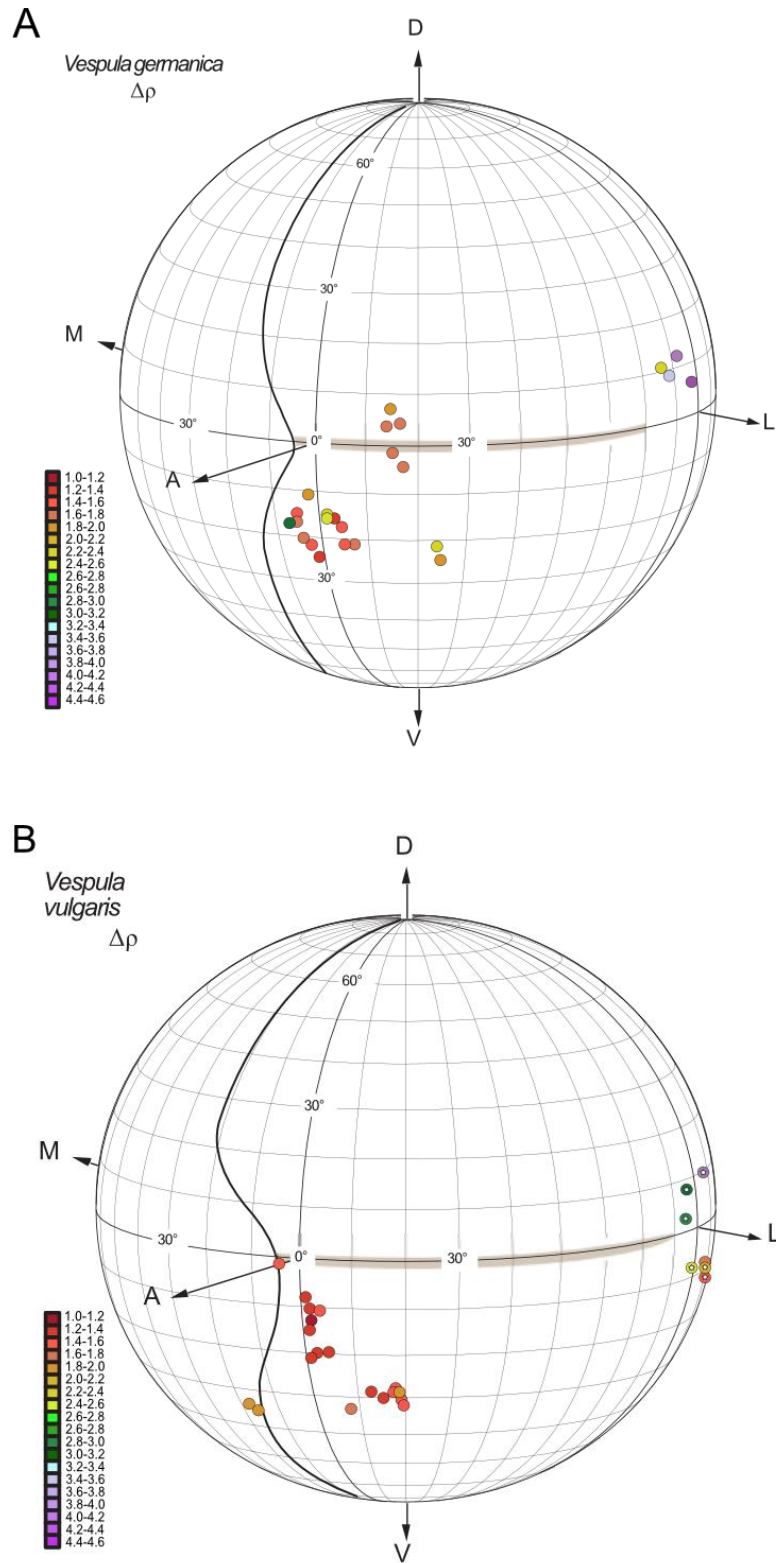
Figure 10. Receptive field widths (acceptance angles *Δρ* in degrees – *colour code*) for *V. germanica* (**A**) and *V. vulgaris* (**B**) plotted on a globe representing the visual field of the left eye (all conventions as in Fig. 6). *Dots with a white centre* represent receptive fields located at a longitude greater than 100°, on the rear-side of the globe.

5.DISCUSSION

In the compound eyes of insects, the density of the ommatidia is represented by the interommatidial angle ($\Delta\varphi$), which defines the anatomical resolution of the eye. If an individual has smaller $\Delta\varphi$, it will potentially have greater spatial resolution (Land 1981; 1999). A possible complicating factor is that these and many other wasps possess an equatorial cuticular "peninsula" that partially divides their compound eyes into dorsal and ventral halves. Even though this peninsula potentially could have created a blind spot in the frontal visual field, we discovered that this was not the case in the two wasps we studied. Interestingly, however, the way that interommatidial angles varied within the eye changed abruptly at this peninsula, with the dorsal half of the eye generally having higher $\Delta\varphi$ and lower spatial resolving power than the ventral half of the eye (Fig. 7).

In general, we found that the average interommatidial angle in wasps decreases smoothly from the dorsal part of the eye to the equator (i.e. from 80° to 0°), and increases smoothly again towards the ventral part of the eye (latitude from 0° to -80°). Although both species exhibited small $\Delta\varphi$ values (Fig. 7), *V. germanica* showed the smallest average $\Delta\varphi$ of 1.0°, at around -10° latitude and between 10 and 20° in longitude. Although *V. vulgaris* has the larger minimum values of $\Delta\varphi$, they are found in an eye region similar to that in *V. germanica*. These small values of $\Delta\varphi$ (Fig. 7), together with larger facets (Fig. 6) and narrower photoreceptor receptive fields at the same location (Figs. 7-9), indicate the presence of an "acute zone" that allows higher spatial resolution in the frontal-ventral part of the eye. The acute zones of these wasps afford a visual performance rivalling the best seen in other day-flying hymenopterans (e.g. the carpenter bees *X. leucothorax and X. tenuiscapa*: Somanathan et al. 2009).

If we compare the photoreceptor receptive fields of *V. germanica* and *V. vulgaris* with some other insects, it is evident that these wasps have reasonably small values of acceptance angle $\Delta\rho$, and thus high spatial resolution. Bees have a frontal acute zone within which photoreceptors have a frontal average acceptance angle of 1.6° (Rigosi et al. 2017a), while other species such as the drone fly *Eristalis tenax* ($\Delta\rho = 0.9°$) (Rigosi et al. 2017b) and the butterfly *Melanitis leda* ($\Delta\rho = 1.5°$) (Land and Osorio 1990) also have similar acceptance angles and consequently similar spatial resolution. However, although *V. germanica* and *V. vulgaris* exhibit low values of $\Delta\rho$, there are other species with ventral-frontal acute zones that house photoreceptors having similar or smaller acceptance angles (and higher spatial resolution), such as the fly *Calliphora erythrocephala* ($\Delta\rho = 1.0°$) (Smakman JGJ et al. 1984),

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Calliphora stygia ($\Delta\rho = 0.9°$) (Rigosi et al. 2017b) and the mantid *Tenodera australasiae* ($\Delta\rho = 0.7°$) (Rossel 1979).

Insects with $\Delta\rho$ less than about 1° are often predators, including dragonflies (Laughlin 1974), mantids (Rossel 1979), killer flies (Wardill et al. 2017) and some wasps (Kelber et al. 2011), among others. These insects typically have a forward pointing acute zone for tracking prey (Land 1997). This offers one explanation for the small $\Delta\varphi$ measured for the wasps used in the present study (Fig. 7). *V. germanica* and *V. vulgaris* are predators and scavengers that seek different types of protein sources (Broekhuizen and Hordijk 1968; Archer 1977; Gambino 1986; Spradbery 1973, 1991; Edwards 1980), including flies, lepidopteran larvae, ants, grasshoppers and arachnids, among others (Harris 1991). Both species hunt from above, and their frontal-ventral acute zones would thus be beneficial for this purpose (Harris et al. 1991; D'Adamo and Lozada 2007). These two species are also known to hunt continuously, seeking food and returning with it to the larvae and individuals that stay in the nest (these species do not store food in the hexagonal chambers of the nest: Reeve and Gamboa 1983). It has been reported (Harris et al. 1991) that in forests where *V. germanica* and *V. vulgaris* coexist, the former forages more on the forest floor and in open areas surrounding the forest while the latter tends to forage more around the foliage of trees and shrubs (Harris et al. 1991). Floral nectar is also an important part of the diet of these wasps and their acute zones are well positioned to view flower structure as the wasps land. Additionally, *V. germanica* and *V. vulgaris* are ground-nesting wasps and the nest entrance is well positioned within the acute zone during landing.

This acute zone found in *V. germanica* and *V. vulgaris* is similar to that found in the nocturnal European hornet (*Vespa crabro*). As in the wasps studied here, this species possesses an acute zone in the frontal-ventral region of the eye, with narrower interommatidial angles and larger facets (Kelber et al. 2011). These similarities between *V. germanica*, *V. vulgaris* and *V. crabro*, can be explained by examining their biology and predatorial behaviour. All three species are predators that hunt from above, in some cases hovering over the prey. However, while *V. germanica* and *V. vulgaris* build their nests at ground level, *V. crabro* builds its nests in higher places. In contrast, the nocturnal sweat bee *Megalopta genalis* has an acute zone aligned along the frontal equator of the eye (Warrant et al. 2004), a location well-suited to the landing behaviour of *M. genalis* at its nest.

Not all insects have evolved acute zones for predation or landing. Some have evolved them in the context of sex. For instance, the males of higher flies (such as *Calliphora*

vicina, Musca domestica and *Eristalis tenax*) have larger eyes than females and exhibit an acute zone in the frontal-dorsal region of the eye (Land 1985, Straw et al. 2006). The females lack such an acute zone. Male flies use this acute zone to chase females for mating and to chase males during territorial fights (Straw et al. 2006). Some butterflies also exhibit such sexual dimorphism in eye design in the context of mating (e.g. *Asterocampa leila*; Rutowski and Warrant 2002). However, whether sexual dimorphism is evident in the eyes of *V. germanica* and *V. vulgaris* workers, queens and drones has not been investigated.

When we calculated the optical sensitivity S of the eyes of the two wasps (at the centre of their acute zones), we found that both have values typical of diurnal insects with apposition compound eyes (Cronin et al. 2014): 0.20 $\mu m^2 sr$ for *V. germanica* and 0.14 $\mu m^2 sr$ for *V. vulgaris*. These values are comparable to those calculated for other diurnal hymenopterans having apposition eyes, for example the carpenter bees *Xylocopa tenuiscapa* and *Xylocopa leucothorax* ($S = 0.3$ and 0.1 $\mu m^2 sr$, respectively: Somanathan et al. 2009), the honeybee *Apis mellifera* ($S = 0.1$ $\mu m^2 sr$: Greiner et al. 2004) and the wasp *Polistes occidentalis* ($S = 0.1$ $\mu m^2 sr$: Greiner 2006). In contrast, the optical sensitivities of nocturnal bees and wasps tend to be around 10-30 times higher, as might be expected of eyes adapted for a life in dim light. Examples include the sweat bee *Megalopta genalis* ($S = 2.7$ $\mu m^2 sr$: Greiner et al. 2004), the carpenter bee *Xylocopa tranquebarica* ($S = 2.7$ $\mu m^2 sr$: Somanathan et al. 2009) and the wasp *Apoica pallens* ($S = 3.0$ $\mu m^2 sr$: Greiner 2006). A notable exception is the nocturnal hornet *Vespa crabro*, which has an optical sensitivity typical of a day-active insect ($S = 0.1$ $\mu m^2 sr$: Kelber et al. 2011). *V. crabro* possibly increases visual sensitivity for nocturnal activity by instead relying on higher neural summation mechanisms.

In conclusion, *V. germanica* and *V. vulgaris* have compound eyes that are typical of visually active diurnal insects. They each possess an acute zone in the ventral-frontal part in the eye, with high spatial resolution subserved by small interommatidial angles and narrow photoreceptor receptive fields (i.e. low acceptance angles), optical properties well suited to the hunting and foraging lifestyles that characterise these two species of wasps.

6. SOCIETAL AND ETHICAL CONSIDERATIONS

V. germanica and *V. vulgaris* are wasps with a huge success as an invasive species. They have colonized numerous countries going from Argentina to New Zealand, impacting the agriculture, economy and fauna and even medical importance due to their aggression. These wasps are social and easy adapted predators that usually become a pest, affecting native species through predation or competition.

Because of this, different research groups have made huge efforts on finding new ways to control these invasive species: like toxic baiting or fire removal. However, although these methods are very efficient, they are not specific to these species and affect a lot of other insects. As mentioned before in this document, several studies focused on their behaviours, but there is a lack of knowledge regarding their cognitive abilities concerning their visual system. It is highly probable that these species of wasps have such a great success as invasive species due to their high visual resolution as well as their high visual acuity.

With the information provided by this project, we can increase our knowledge of these species and thus, create new ways to deal with them in the countries where they have become a pest or create new ways to protect them in Europe, from where they originally are. On the other hand, another important ethical consideration while working with wasps (or insects) is the experiment regulations. It is commonly debated if insects should be regulated for being sentient animals able to feel pain. Some studies showed that insects exhibit "discomfort" behaviours, but not pain. Although this is a topic that is still under a lot of research, it is very difficult to demonstrate if they are or are not able to feel pain. Scientists proved that vertebrates feel pain with verbal reports (sound) and behavioural demonstrations (e.g. protecting a burned paw/hand or avoiding a hot surface). Besides this, vertebrates possess nociceptors (pain receptors), which are activated when pain is inflicted (nociceptive stimuli). Nevertheless, insects do not react to nociceptive stimuli or any behavioural demonstration when pain is inflicted on them. It has been shown that insects may exhibit some "discomfort" behaviours, but not related to pain.

We cannot completely assume that they do not feel pain. We should only assume that they do not perceive pain the same way that vertebrates do. Usually, there are no strict regulations while working with insects, because they are not considered to be conscious or sentient animals, but as scientists, we should consider this when doing research involving insects.

7. ACKNOWLEDGMENTS

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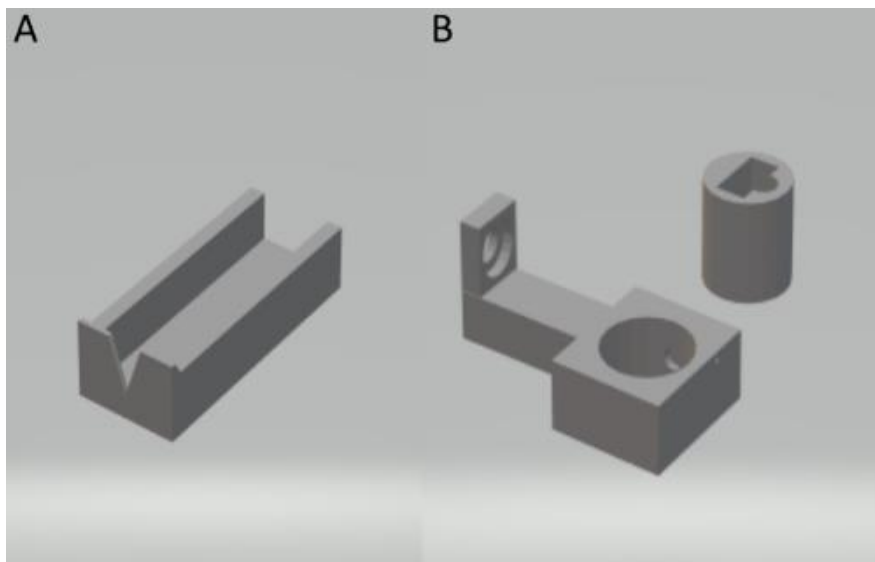Warrington, E. K. (1996). *The Camden memory tests manual* (Vol. 1). Psychology Press.

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9. APPENDIX
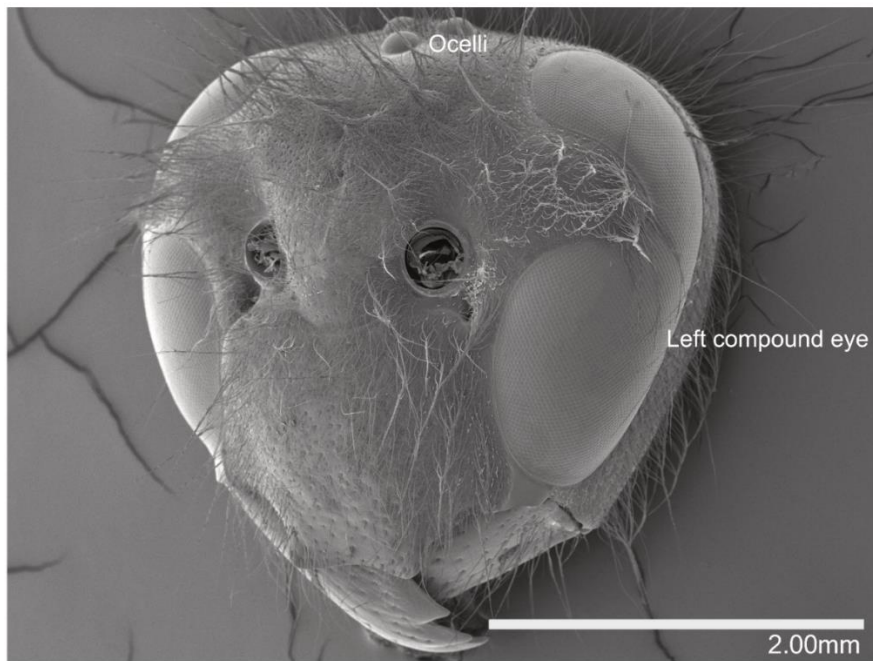
Figure A1. Scanning electron microscope image of the head of *V. germanica* showing the ocelli and the compound eye.

Figure A2. 3D model of the holder used for holding and immobilizing wasps during the injection and optics procedure (**A**), and the holder attached to the goniometer (**B**) that allowed movement of the wasps in the three movement axes (pitch, yaw and rotation).