Large variation among photoreceptors as the basis of visual flexibility in the common backswimmer

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The common backswimmer, Notonecta glauca, uses vision by day and night for functions such as underwater prey animal capture and flight in search of new habitats. Although previous studies have identified some of the physiological mechanisms facilitating such flexibility in the animal’s vision, neither the biophysics of Notonecta photoreceptors nor possible cellular adaptations are known. Here, we studied Notonecta photoreceptors using patch-clamp and intracellular recording methods. Photoreceptor size (approximated by capacitance) was positively correlated with absolute sensitivity and acceptance angles. Information rate measurements indicated that large and more sensitive photoreceptors performed better than small ones. Our results suggest that backswimmers are adapted for vision in both dim and well-illuminated environments by having open-rhabdom eyes with large intrinsic variation in absolute sensitivity among photoreceptors, exceeding those found in purely diurnal or nocturnal species. Both electrophysiology and microscopic analysis of retinal structure suggest two retinal subsystems: the largest peripheral photoreceptors provide vision in dim light and the smaller peripheral and central photoreceptors function primarily in sunlight, with light-dependent pigment screening further contributing to adaptation in this system by dynamically recruiting photoreceptors with varying sensitivity into the operational pool.

1. Introduction

The visual system of Notonecta glauca, the common backswimmer, must provide for numerous challenges arising from the insect’s diverse visual environments and behavioural demands. Backswimmers prefer to live in freshwater ponds, preying upon aquatic organisms as well as terrestrial insects of suitable size that have fallen into the water. They are active around the clock, and use their vision for underwater hunting as well as during flight in search of new habitats. Because of its interesting behaviour and ecology [1–4], the backswimmer’s vision has been broadly studied.

The backswimmer compound eye is an acone-type open-rhabdom apposition eye with corneal structure optimized for creating sharp images in both air and water [5–7]. There are two zones of high acuity in each eye, ventral and dorsal, with 75% of ommatidia viewing a binocular visual field [6]. Each ommatidium consists of two fused central rhabdomeres surrounded by a ring of six detached different-sized peripheral rhabdomeres [8], and an adjustable pigment aperture is present in front of the rhabdom [9]. Rhabdomeres of two central photoreceptors are situated close to each other, with one of them located more proximally than the other, as in the fly [8]. Microspectrophotometric studies found three visual pigments in the rhabdomeres: peripheral rhabdomeres contain a pigment with an extinction maximum at 560 nm (coinciding with the intensity maximum of scattered background light in turbid phytoplankton-rich water [10]) and with sensitivity to red light, whereas...
central rhabdomeres contain pigments with extinction maxima at 345 and 445 nm.

Very large differences in environmental light intensities between day and night require different visual adaptations. Many distinct adaptations have been described in the compound eyes of nocturnal and diurnal insects, which help to optimize visual performance to their respective ecological niches. These include specializations in general eye design (apposition versus superposition eye), lens and rhabdom size, structure of the rhabdom (fused versus open rhabdom), membrane dynamic filtering, transduction gain and neuronal organization in the lamina [11–15]. Under low light conditions, vision must be sufficiently sensitive to extract information from dimly illuminated environments, while also dealing with relatively high photon noise. Adaptations to nocturnal vision generally aim to improve sensitivity to light and reliability of phototransduction, usually at the expense of visual speed. In bright light, by contrast, photoreceptors must be able to accommodate large changes in intensity produced by shading, highlights and the sun glinting off objects, for example, without excessive saturation of the phototransduction cascade and voltage responses. They also have to reliably resolve small contrasts to make best use of the high SNR afforded by abundant photons.

To deal with diurnal changes in illumination, many animals that use vision around the clock alter the structure of their compound eyes, adapting the optics to prevent the large numbers of microvilli that must be used at night to trap scarce photons from making excessive metabolic demands during the day [16–18]. For instance, locusts and *Limulus* rebuild their rhabdomes every day, with profound modifications in photoreceptor biophysics, position and pigment cell geometry [19–21]. Indeed, *Notonecta*’s eye is remarkably similar to the eye of another aquatic predator that flies from pond to pond, the giant Asian water bug *Lethocerus* [22], and to that of *Tipulid* flies [17,23]. Like *Notonecta*, these species have an open (or partially open) rhabdom which moves closer to the lens at night to increase the rhabdomeres’ angular subtense, and then back to the focal plane during the day to narrow this angle. Photoreceptor movements are accompanied by pigment cell movements that open the entrance aperture at night and close it during the day, when the pigment also shields the outer rhabdomeres. This type of eye is very effective at trading sensitivity for angular resolution in scotopic conditions with a subjective ‘night’ period matching the actual day (regimen 2; see below for details). Photoreceptor responses were recorded using microelectrodes (aluminosilicate glass; Harvard Apparatus, UK) manufactured with a laser puller (P-2000; Sutter Instrument, USA) and filled with 2 M KCl solution to a final resistance of 80–110 MΩ. All cells had resting potentials of −44 mV or lower (the average resting potential was $-54.9 \pm 8.6$ mV; n = 23); if photoreceptor condition deteriorated during recordings to give a resting potential of −44 mV or higher, voltage recordings were discontinued.

2. Material and methods

(a) Patch-clamp recordings from photoreceptors in dissociated ommatidia

Adult backswimmers (*N. glauca*) were collected locally (Oulu, Lund) or purchased from Blades Biological (UK). Dissociation of ommatidia (electronic supplementary material, figure S1) and electrophysiological recordings were performed as described previously [26–28]. In brief, an Axopatch 1-D patch-clamp amplifier and CLAMP v. 9.2 software (Axon Instruments/ Molecular Devices, CA, USA) were used for data acquisition and analysis. Patch electrodes (made from borosilicate glass; World Precision Instruments, Sarasota, FL, USA) had resistance of 3–8 MΩ. Bath solution contained (in mM): 120 NaCl, 5 KCl, 4 MgCl₂, 1.5 CaCl₂, 10 N-Tris(hydroxymethyl)-methyl-2-amino-ethanesulfonic acid (TES), 25 proline and 5 alanine, pH 7.15. Patch pipette solution contained (in mM): 140 KCl, 10 TES, 2 MgCl₂, 4 Mg-ATP, 0.4 Na-GTP and 1 NAD, pH 7.15. All chemicals were purchased from Sigma Aldrich (St. Louis, MO, USA). The liquid junction potential (LJP) between the bath and the normal intracellular solution was $-4$ mV. All voltage values cited in text were corrected for the LJP. Series resistance was compensated by 80%. All cells had resting potentials of $-44$ mV or lower (the average resting potential was $-54.9 \pm 8.6$ mV; n = 23); if photoreceptor condition deteriorated during recordings to give a resting potential of $-44$ mV or higher, voltage recordings were discontinued.

(b) In vivo intracellular single-electrode recordings

In vivo intracellular single-electrode recordings were performed as described previously [29]. In brief, for intracellular recordings, backswimmers were maintained in reversed 12–12 illumination conditions with a subjective ‘night’ period matching the actual day (regimen 2; see below for details). Photoreceptor responses were recorded using microelectrodes (aluminosilicate glass; Harvard Apparatus, UK) manufactured with a laser puller (P-2000; Sutter Instrument, USA) and filled with 2 M KCl solution to a final resistance of 80–110 MΩ). Signals were amplified with an intracellular amplifier (SEC-05L; NPI, Germany). Before recording the angular sensitivity, the optical axis of the photoreceptor was determined by changing the polar angles of the light source while recording light responses in a Cardan-arm system. Then the V-log(I) curve was plotted to obtain the stimulus intensity eliciting the half-maximum response. This light intensity was used to measure the receptive field of the photoreceptor by changing the polar angle. Voltage responses, measured at different angles, were converted to angular sensitivity after the correction for the azimuthal angle. The angular diameter of the light source was approximately 1°.

Recordings were performed mainly but not exclusively from the central part of the eye; no notable differences in photoreceptor properties were observed between different parts of the eye. All cells used for analysis had resting potentials of $-45$ mV or lower, showed single angular sensitivity peaks and demonstrated transient depolarization with the zero attenuation filter of at least 22 mV in amplitude (at least 35 mV at the maximum light intensity); in addition, in current-clamp experiments involving current injections to determine capacitance, only cells showing strong rectification without any recording artefacts were used to ensure that capacitance was not overestimated due to electrode blockade.

Light stimulation was performed as described previously [28]. In brief, a computer-controlled custom-made voltage-to-current driver for light-emitting diodes (LEDs) was used to drive 10 (in patch-clamp experiments) or 13 (in intracellular experiments) monochromatic LEDs (Roithner Laser Technik, Austria), covering a range from 355 to 639 nm, which were used in combination with neutral density filters (Kodak, New York, NY, USA). In...
patch-clamp experiments, full-field stimuli were delivered to dissociated ommatidia through the inverted microscope objective.

(c) Pupil sizes and rhabdomere migration
For experiments with pseudopupil adjustment and rhabdomere migration, half the population was maintained under normal 12–12 illumination conditions (regimen 1), whereas the rest lived in regimen 2 so that animals in both night and day pupil adaptation states [2] could be studied during day-time. To test adaptation to bright sunlight, the regimen 1 animals were placed in direct sunlight for 3 h. To obtain fully dark-adapted animals, another sample of backswimmers maintained under regimen 2 was placed in a dark box for the same period. Animals adapted to laboratory light were obtained in similar way. Following the exposure, backswimmers were killed and their heads were fixed in 4% paraformaldehyde in phosphate-buffered saline supplemented with 3% sucrose. After rinsing, dehydration and embedding in Spurr’s embedding medium, 4 µm sections were cut and mounted with Entellan (Merck, Germany).

(d) Data analysis
Information rates were analysed using the Shannon method [30] as described previously [28,31]. In brief, to measure photoreceptor performance, a computer-generated 90 s light contrast series, made of 30 s long steady light pre-pulse followed by a test pulse consisting of 30 repetitions of a 2 s Gaussian randomly modulated (white-noise, WN) sequence with a cut-off frequency of 200 Hz was used to obtain information rates (IRs). Analysis of responses to WN stimulation was performed by estimating the 2 s signal $S(f)$ by averaging voltage responses to thirty 2 s long segments of the stimulus. The noise was then obtained by subtracting the signal estimate from the original (noise-containing) sequences. The signal-to-noise ratio (SNR($f$)) was obtained in the frequency domain as $\text{SNR}(f) = |S(f)|^2/|N(f)|^2$, where $|S(f)|^2$ is the estimated response signal power spectrum and $|N(f)|^2$ is the estimated noise power spectrum. The contrast gain of voltage responses $|T(f)|$ was calculated by dividing the cross-spectrum of photoreceptor input (WN contrast, $C(f)$) and output (photoreceptor signal) $S(f)C^*(f)$ (the asterisk denoting complex conjugate) by the autospectrum of the input $C(f)C^*(f)$ and taking the absolute value of the resulting frequency response function $T(f)$: $|T(f)| = |S(f)C^*(f)/C(f)C^*(f)|$. The Shannon IR for WN modulated responses was calculated according to the equation $\text{IR} = \int [\log_2(|S(f)|/|N(f)| + 1)]df$ within a frequency range from 1 Hz to a frequency where the value of SNR equalled 0.5.

All values are means ± s.d. Spearman’s rank correlation coefficient ($\rho$) was calculated as described previously [32]. Spearman’s $\rho$ was considered significantly different from zero when the p-value calculated by using the large-sample approximation test in MATLAB was less than 0.05.

3. Results
(a) General properties of backswimmer photoreceptors
Basic features of photoreceptor function were first investigated with the patch-clamp method in isolated ommatidia. Figure 1a shows examples of action spectra obtained in patch-clamp recordings. Of 34 photoreceptors, two cells demonstrated responses to UV light only (figure 1a, represented by violet trace); two cells had a response maximum at 435 nm (blue trace); one cell had a response maximum at 470 nm (cyan trace); the remaining cells exhibited strongest responses to LEDs with maxima between 490 and 591 nm (green and dark yellow traces), and with an average response maximum at 535 nm. Figure 1b shows examples of microscopic current responses, the quantum bumps elicited by 1 ms flashes of light with enough intensity to evoke responses with approximately 50% success rate. The average quantum bump amplitude was $−47.2 ± 19.2 \text{ pA} (n = 26)$. In patch-clamp, voltage bumps were present in all photoreceptors at rest upon stimulation by steady low-intensity light. Voltage bump amplitude usually varied from 0.5 to 2 mV.

Figure 1c shows macroscopic voltage and light-induced current (LIC) responses to a naturalistic stimulus. Figure 1d quantifies transient and sustained depolarization during

![Figure 1](image-url)
light responses of dark-adapted photoreceptors to steady light of increasing intensity in patch-clamp and intracellular experiments. The differences in depolarization amplitudes were apparently caused by dissimilar illumination conditions (side-on stimulation of dissociated ommatidia in vitro versus natural illumination through optics with intact pigment screening in intracellular experiments).

Consistent with previous studies documenting significant differences in the size of individual rhabdomeres [8], backswimmer photoreceptors exhibited large variation in whole-cell capacitance. Capacitance was measured both in vitro, in voltage-clamp experiments (patch-clamp) by calculating the charge under the capacitive transient following a small voltage step, and in vitro, in intracellular current-clamp experiments involving current injections (figure 1c), from the time constant. In patch-clamp experiments, whole-cell capacitance varied from 107 to 907 pF with a median of 247 (190–369) pF (interquartile range) (n = 38), whereas intracellular recording capacitance varied from 262 to 1120 pF with a median of 379 (303–507) pF (n = 36) (figure 1f).

In Notonecta photoreceptors, the depolarizing effects of LIC were opposed by two K+ currents, a rapidly activating and inactivating (I\textsubscript{A}), and a very slowly inactivating delayed rectifier current (I\textsubscript{DR}) (electronic supplementary material, figure S2). I\textsubscript{DR} showed almost no inactivation within the physiological voltage range (approx. between −70 and −20 mV). I\textsubscript{A} was sensitive to 4-AP and in most cells could be completely abolished by a 2 s inactivating pre-pulse to −34 mV. Peak conductances and half-activation potentials (V\textsubscript{1/2}, determined by fitting the data points with a Boltzmann function) of these currents were of similar magnitude (electronic supplementary material, figure S2d). The values of V\textsubscript{1/2} were −25.2 ± 3.7 mV for I\textsubscript{A} and −23.8 ± 6.3 mV for I\textsubscript{DR} (n = 7).

(b) Absolute sensitivity
We found a large variation in absolute sensitivity among green photoreceptors. Sensitivity was measured in patch-clamp experiments by counting voltage bumps evoked by continuous stimulation at light intensities dim enough to elicit less than 10 bumps s\textsuperscript{−1} (less than 5 bumps s\textsuperscript{−1} on average) in order to avoid overlapping bumps. For each photoreceptor, sensitivity to light was defined as a reciprocal of the light intensity that would induce 1 effective photon s\textsuperscript{−1} (i.e. 1 bump s\textsuperscript{−1}). The relative light sensitivity was then calculated as a fraction of sensitivity of the most sensitive cell in the sample. Strong correlation was found between sensitivity and the corresponding capacitance value (ρ = 0.76, p = 0.00011; figure 2).

(c) Photoreceptor performance in bright light
To evaluate photoreceptor performance and information processing, we used a 90 s WN contrast sequence to stimulate photoreceptors over a range of light intensities from a sensitivity threshold to a saturating intensity (figure 3). Responses to WN modulated light are shown in figure 3. Photoreceptor depolarization increased, bump noise decreased and contrast resolution improved with increasing light intensity, up to the level of bright light (around 400 000 effective photons s\textsuperscript{−1} in figure 3d). Consistent with these changes, contrast gain and information rate also increased (figure 3bc). The maximal gain was 15.0 ± 4.4 mV per unit of contrast with an average 50% cut-off frequency of 14.1 ± 5.0 Hz (n = 11). The maximal average IR (IR\textsubscript{max}) was 87 ± 42 bits s\textsuperscript{−1} (n = 23). In still brighter light, photoreceptor performance deteriorated again, reflecting saturation of transduction units in bright light as a consequence of stimulating dissociated ommatidia deprived of optical adaptation mechanisms [33,34]. This decrease in the IR in very bright environments is very similar to that observed in photoreceptors of dissociated stick insect, cricket and water strider ommatidia [28,31,35], and consistent with the saturation effects observed in white-eyed blowflies and Drosophila [36,37].
The functional importance of photoreceptor size and its variation, already demonstrated in the correlation between sensitivity to light and whole-cell capacitance (figure 2), was supported by two additional observations: larger and more sensitive backswimmer photoreceptors had higher IR_{max} values than their smaller and less sensitive counterparts (ρ = 0.57, p = 0.0045 for the correlation between capacitance and IR_{max} n = 23 cells; ρ = 0.70, p = 0.0024 for the correlation between the relative sensitivity and IR_{max} n = 16 cells; figure 3d,e), which might be a useful development considering the relatively small number of photoreceptors capable of seeing in very dim environments.

**Figure 4.** Cell size, angular sensitivity and absolute sensitivity in vivo. (a) Examples of normalized angular sensitivity functions of different photoreceptors; dotted line shows the 50% voltage response level at which the acceptance angle was determined. (b) Distribution of acceptance angle values. (c) Correlations between photoreceptor capacitance and acceptance angle for two experimental samples at different light intensities (see Results). (d) Variation in steady-state depolarization responses (n = 21) with definition of thresholds used to infer absolute sensitivities. (e) Correlations between acceptance angle and the light intensities at which steady-state depolarization reaches either 0.5 or 1 mV. (f) Correlations between acceptance angles and plateau voltage at two light intensities (attenuations ‘–2’ and ‘–3’).

As in patch-clamp experiments, sensitivity varied more than 100-fold, depending on the threshold used. Also, there were significant correlations between the acceptance angle and steady-state depolarization at a specified light intensity threshold (ρ = 0.73, p = 0.0002 for the attenuation ‘–3’; and ρ = 0.49, p = 0.025 for the attenuation ‘–2’, n = 21; figure 4f). However, it should be noted that the depolarization level depends on bump frequency, quantum bump size, membrane gain (determined by input resistance, affected, for example, by the voltage-activated conductances), voltage-dependence of gain (determined by voltage-dependencies of ion channels) and voltage bump amplitude. Because all these parameters may vary from cell to cell, these steady-state depolarization metrics probably underestimated the correlation between acceptance angles and absolute sensitivity of backswimmer photoreceptors.

**Central rhabdomeres**

Two central photoreceptors in each ommatidium contain either a UV- or a blue-sensitive pigment [38]. Because they seem to

(d) In vivo recordings: capacitance, angular sensitivity and absolute sensitivity

While patch-clamp experiments allow characterization of biophysical properties of photoreceptors without the bias introduced by ommatidial optics, functional interpretation of the discovered variations in the key properties of backswimmer photoreceptors (capacitance, absolute sensitivity and performance) is compromised by the fact that our patch-clamp preparation required side-on illumination, which is non-physiological for photoreceptors, and does not take into account angular sensitivity.

To determine whether larger photoreceptors, characterized by higher absolute sensitivity in the in vitro experiments, have higher absolute and angular sensitivities than their smaller counterparts in vivo, intracellular recordings were performed from backswimmers maintained under reverse illumination conditions (to maximize the expected variation in acceptance angles). The above-cited capacitance values (figure 1f) calculated from voltage responses tended to be higher than capacitance values obtained in voltage-clamp experiments, possibly due to underestimation of the cellular resistance, caused by an electrode-induced leak, leading to overestimation of capacitance. Also, in vitro capacitance can be underestimated due to the loss of axonal membrane or space-clamp error. However, as anticipated on the basis of the patch-clamp recordings and previous hypotheses, there was large variation in acceptance angle values among photoreceptors (figure 4a,b), from 2.88° to 12.43° with a median of 4.9° (4.0–6.0°) (n = 41). A moderate positive correlation was found between capacitance and acceptance angle values (ρ = 0.45, p = 0.038, n = 21; figure 4c, open circles). A slightly higher correlation was found in a different sample with a 10-fold higher stimulus intensity, which produced wider acceptance angles (ρ = 0.61, p = 0.01, n = 17; figure 4c, closed circles).

To determine the absolute sensitivity in intracellular recordings, we first attempted to calculate it directly, by counting voltage bumps, as in patch-clamp experiments. However, it was not feasible to resolve individual bumps regularly enough due to the relatively high noise and the small bump amplitude. Therefore, we used a more indirect measure of steady-state depolarization in dim light. Figure 4d demonstrates the experimental steady-state depolarization curves with red dashed lines illustrating the types of thresholds that we used to determine the correlation between acceptance angles and absolute sensitivity. Statistically significant positive correlations were found between acceptance angle and light intensity at which the photoreceptor depolarized by 0.5 or 1 mV (the values were obtained from fitted parameters; correspondingly, ρ = 0.63, p = 0.0024 and ρ = 0.61, p = 0.0034, n = 21; figure 4e).
perform a different function than the green peripheral photoreceptors, being responsible for colour vision (e.g. [39]), we compared their electrophysiological properties. Only five UV-, violet- and blue-sensitive photoreceptors were successfully patched, due to their difficult position within the ommatidium. No differences between green photoreceptors and the small sample of UV and blue cells were found in terms of capacitance, ionic currents or information rates. Three intracellular recordings of blue cells were made from intact retinas. All were characterized by relatively small acceptance angles (around 5°) and low absolute sensitivities.

(f) The pupil in light microscopy
To see which photoreceptors receive light in different illumination conditions, we used light microscopy to examine backswimmer retinas adapted to different conditions. Anatomically, it appears that in bright light (figure 5a,d), screening pigment acts as a pupil and may restrict light to the small central rhabdomeres, whereas in dark-adapted eyes (figure 5c,e) the pigment moves away so that light can enter all rhabdomeres. Specifically, in the light-adapted eye, a light-guiding tract about 5–8 μm in diameter and 10 μm long is formed between heavily pigmented pigment cells (figure 5d). In the dark-adapted state, the tract is completely absent and the rhabdomeres are about 40 μm closer to the cornea than in the day-adapted eye, and indent into the cone cells (figure 5a). These movements may have an intrinsic diurnal rhythm as well, as can be seen in the intermediate position of the pigments in the dark-adapted state during subjective day when compared with the dark-adapted pupil during subjective night (figure 5b,e). Adjustment of the pupil implies that the central and peripheral rhabdomeres have performance optima at different light levels.

4. Discussion
In this work, we studied the electrophysiological properties of backswimmer photoreceptors in an attempt to determine the mechanisms that allow the animal to use vision under dramatically different illumination conditions, such as those experienced during day and night. Our results, documenting large variations among individual photoreceptors in capacitance, absolute sensitivity to light and angular sensitivity, with substantial positive correlations between these properties, are fully consistent with the previously reported variation in the size of individual rhabdomeres in backswimmer ommatidia [5,8]. Although the general anatomical design of the backswimmer eye is optimized for vision in well-lit backgrounds (open-rhabdom apposition eye), the presence in each ommatidium of two large peripheral green-sensitive rhabdomeres with wide acceptance angle (the angle subtended by the photoreceptor entrance aperture), and hence a small F-number, and with relatively high absolute sensitivity, can be considered as an adaptation for vision in the dark. One puzzling issue was the apparent scarcity of very large photoreceptors in our experiments. A reason for that could be that large peripheral photoreceptors differ in rhabdom length (and therefore capacitance), or that there is variation in photoreceptor size across the eye resulting in relatively smooth experimental capacitance distribution.

As the large variation in absolute sensitivity and its correlation with capacitance are central to our argument, these findings must answer three main questions: (i) whether the variation in absolute sensitivity and its correlation with capacitance are real; (ii) whether the variation in absolute sensitivity has any functional importance; and (iii) whether it is possible to explain variation in absolute sensitivity largely or fully by variation in other presently determined factors (e.g.
that (i) sensitivity of the diurnal findings reported in this article.

Without exception. These results reinforce the validity of photoreceptors very much like the highly sensitive photoreceptors in the backswimmer (and in the cockroach) clear from these data that some green-sensitive peripheral receptor sensitivity ranges of the backswimmer and cockroach than those for the water strider and stick insect. Therefore, it is observed strong correlations between these two photoreceptor properties in different insect species. In this regard, it is interesting that in all species presented in figure 6, the variations in sensitivity are similarly proportional to the variations in capacitance: if we quantify each correlation by first obtaining the interquartile ranges for both sensitivity and capacitance, and then divide the interquartile range for absolute sensitivity by that for the capacitance variation, the resulting ratios are similar: 0.0072 for the cockroach, 0.0088 for the backswimmer, 0.0092 for the stick insect and 0.0139 for the water strider (although in the latter case there were two classes of peripheral photoreceptors, blue and green, characterized by different absolute sensitivities and average capacitances) [31].

Our estimates made from micrographs of backswimmer ommatidia [8] indicate that the cross-sectional area of the largest pair of rhabdoms is three times greater than that of the smallest pair. However, in vivo sensitivity depends, among other factors, on the product of two probabilities: the probability of a photon entering the rhabdom (which increases with the cross-sectional area of the rhabdom, especially at the distal end) and the probability that rhodopsin absorbs a photon entering the rhabdom (which depends on the length of the rhabdom). In dark, the latter probability seems to be more important, while in bright light it is the entrance aperture size that determines sensitivity. As sensitivity is proportional to the rhabdom membrane area, the threefold difference between the largest and smallest rhabdomeres means that either the wide rhabdomeres should be substantially longer than their narrow counterparts (to accommodate more microvilli) or that other factors, such as those listed above, should disproportionately boost the sensitivity of the largest rhabdomeres or reduce the sensitivity of the smallest ones.

Backswimmers generally prefer to swim or rest on the water surface, often in direct sunlight. To prevent photoreceptor saturation in such conditions, their light attenuation mechanisms must be sufficiently strong. One such mechanism is the dynamic screening by pigment cells situated at the distal end of the ommatidium and forming the pupil [2]. Figure 5 shows cross-sections of the backswimmer pupil in three adaptation states: dark-adapted during night, light-adapted to bright laboratory illumination during the day and light-adapted to sunlight. The pigment diaphragm is fully open in the dark, partially closed in laboratory light and completely shields the peripheral rhabdomeres in sunlight. Note that the pigment cell movement was accompanied by proximal migration of rhabdomeres (figure 5d,e). These observations on rhabdomere and pigment cell movement mirror findings made in the giant water bug Lethocerus [41]. The sensitivity range of the backswimmer pseudopupil is very large, about 7 log units during the day and 6 log units during night, with the total range reaching 8 log units [2]. For comparison, the diurnal blowfly has a pupil dynamic range of 3 log units [34], and the strictly nocturnal moth Hydracaea micacea has a range of 4 log units [42]. Obviously, such a large range reflects the need to use vision both during the day and at night in the backswimmer. Interestingly, shielding of peripheral photoreceptors not only protects the peripheral photoreceptors from bright light, but also enables them to function at otherwise saturating backgrounds, as evidenced by the finding that peripheral

Figure 6. In vitro absolute sensitivity in species with different visual ecologies. Correlations between absolute sensitivity and capacitance are shown for four insects: N. glauca, the stick insect C. morosus [28], the water strider G. lacustris [31] and the American cockroach P. americana; data are presented for adult specimens only; in the case of P. americana, the same dataset was used to extract sensitivity values as published by Immonen et al. [40]. Data are presented in terms of bumps s$^{-1}$ at the light level corresponding to the level –7 in figure 1d.

photoreceptor capacitance). Our results suggest that the answers to these questions are respectively ‘yes’, ‘yes’ and ‘no’.

Figure 6 shows N. glauca absolute sensitivity–capacitance correlation alongside identical data obtained by us previously in three other, not very closely related species in patch-clamp experiments under the same conditions, which to the best of our knowledge constitute the only data of this kind on invertebrate photoreceptors available in the literature. Importantly, all these species have different visual ecologies: the stick insect Carausius morosus is strictly nocturnal, the water strider Gerris lacustris is strictly diurnal, and the American cockroach Periplaneta americana is active during twilight and night-time. All these species demonstrate strong positive correlations between absolute sensitivity and capacitance [28,31] ($P$. americana sensitivity and capacitance values were obtained from the dataset as published by Immonen et al. [40]). In fact, this was found in all insect species where we addressed this issue, without exception. These results reinforce the validity of findings reported in this article.

Regarding the second question, it is apparent from figure 6 that (i) sensitivity of the diurnal G. lacustris is much lower than that of the nocturnal C. morosus, (ii) sensitivity ranges for both the water strider and the stick insect are substantially smaller than those for the backswimmer and cockroach, and (iii) photoreceptor sensitivity ranges of the backswimmer and cockroach exceed those of the water strider and stick insect. Therefore, it is clear from these data that some green-sensitive peripheral photoreceptors in the backswimmer (and in the cockroach) function very much like the highly sensitive photoreceptors of the nocturnal stick insect, whereas others have much lower sensitivity like those observed in the water strider. This pattern implies that the variation in absolute sensitivity in N. glauca is of functional importance.

However, absolute sensitivity varied more than 100-fold, while capacitance varied approximately 10-fold. Considering the obviously linear relationship between absolute sensitivity and capacitance, the variation in capacitance alone can apparently explain only a minor fraction of the variation in sensitivity. This discrepancy suggests that some other factors regulating absolute sensitivity (e.g. photosensitive and screening pigment densities, rhodopsin/metarhodopsin equilibrium, quantum efficiency, microvillus recovery periods, etc.) may also positively or negatively correlate with photoreceptor size, which might explain why we have consistently observed strong correlations between these two photoreceptor properties in different insect species. In this regard, it is interesting that in all species presented in figure 6, the variations in sensitivity are similarly proportional to the variations in capacitance: if we quantify each correlation by first obtaining the interquartile ranges for both sensitivity and capacitance, and then divide the interquartile range for absolute sensitivity by that for the capacitance variation, the resulting ratios are similar: 0.0072 for the cockroach, 0.0088 for the backswimmer, 0.0092 for the stick insect and 0.0139 for the water strider (although in the latter case there were two classes of peripheral photoreceptors, blue and green, characterized by different absolute sensitivities and average capacitances) [31].
photoreceptors can mediate optomotor reactions even in bright light, when they are shaded by the pigment diaphragm [25]. (Although the attenuation strength of shielding pigment filtering in the backswimmer is not known it might be similar to that in diurnal flies, i.e. between 2 and 3 decades [33].) The proximal rhodomere migration should also act as a pupil, providing additional attenuation, although its strength was not evaluated in this study.

Overall, combining previous studies with our results suggests that the backswimmer’s retina can be functionally separated into at least two photoreceptor subsystems. The first would consist of the largest and most sensitive peripheral photoreceptors. These are sensitive to green and allow the backswimmer to see in dim light. The second subsystem would consist of the smaller peripheral and central photoreceptors, and would be used for vision in bright light and during flight. Even though the operational ranges of these subsystems are expected to overlap substantially, such a classification could be merited by consistent differences in several respects: large green-sensitive photoreceptors are generally also more sensitive to light, and have larger acceptance angles, slower membrane responses and higher $IR_{\text{max}}$ than the smaller photoreceptors. Even though little is known about the properties of central photoreceptors, it appears that they are similar to the properties of peripheral ones, but because pigment screening cannot shield them even in the brightest light, they should have lower sensitivity to light than peripheral photoreceptors in order to avoid saturation in sunlight. In this context, it is interesting that the rhodome of one of the two central photoreceptors is always situated proximally to the other [8], which necessarily entails lower sensitivity to light due to a lower photon flux. This raises the possibility that the functions of the central rhodomeres are also optimized to different backgrounds—the proximal rhodomere may continue to function when the distally situated rhodomere is already saturated.

**Data accessibility.** The data supporting the article are available in the electronic supplementary material.

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# References


