Not flying blind: A comparative study of photoreceptor function in flying and non-flying cockroaches

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Summary statement: Photoreceptors of flying Panchlora and non-flying Periplaneta cockroaches are compared using imaging and electrophysiological methods. The findings are discussed in relation to the visual ecological paradigm developed for Diptera.
ABSTRACT

Flying is often associated with superior visual performance since good vision is crucial for detection and implementation of rapid visually-guided aerial movements. To understand the evolution of insect visual systems it is therefore important to compare phylogenetically related species with different investments into flight capability. Here, we describe and compare morphological and electrophysiological properties of photoreceptors from the habitually flying green cockroach *Panchlora nivea*, and the American cockroach *Periplaneta americana*, which flies only at high ambient temperatures. In contrast to *Periplaneta*, ommatidia in *Panchlora* were characterized by two-tiered rhabdom, which might facilitate detection of polarized light while flying in the dark. In patch-clamp experiments, we assessed the absolute sensitivity to light, elementary and macroscopic light-activated current and voltage responses, voltage-activated potassium (Kv) conductances, and information transfer. Both species are nocturnal, and their photoreceptors were similarly sensitive to light. However, a number of important differences were found, including the presence in *Panchlora* of a prominent transient Kv current and a generally low variability in photoreceptor properties. The maximal information rate in *Panchlora* was one-third higher than in *Periplaneta*, due to a substantially higher gain and membrane corner frequency. The differences in performance could not be completely explained by dissimilarities in the light-activated or Kv conductances; instead, we suggest that the superior performance of *Panchlora* photoreceptors mainly originates from better synchronization of elementary responses. These findings raise a question whether evolutionary tuning of photoreceptor properties to visual demands proceeded differently in Blattodea than in Diptera.
INTRODUCTION

Visual systems of fast-flying insects generally outperform those of slow flyers and crawlers. In several species of flies photoreceptor performance was linked to specific visual ecological aspects of lifestyle and behaviour (Niven et al., 2007; Weckström and Laughlin, 1995). In general, airborne predators possess large compound eyes with photoreceptors capable of resolving contrast changes with outstanding precision and speed. Temporal resolution of photoreceptors of the top diurnal fliers is extremely high, with membrane corner frequencies exceeding 100 Hz (Laughlin and Weckström, 1993). Such photoreceptors are also characterized by relatively low absolute sensitivity due to diurnal lifestyle, fast light adaptation, low input resistance, and low membrane gain. The two latter properties are caused by large, rapidly activating, sustained voltage-activated potassium (Kv) conductances (Laughlin and Weckström, 1993; Weckström and Laughlin, 1995). However, the fitness costs of adaptations underlying superior performance, and especially that of leaky membranes, can be high (Niven and Laughlin, 2008), preventing widespread occurrence of such features. As a consequence, it was shown that photoreceptors of slow fliers tend to express a more inactivating Kv conductance with a small sustained component, and therefore have relatively high input resistance, high membrane gain, and low membrane corner frequency.

Although similar adaptations can be expected in other flying insects, this problem was until recently not addressed beyond Diptera. We recently examined Kv conductances in fifteen phylogenetically diverse species from several orders (Frolov et al., 2016). We found that rapid diurnal flyers indeed express large non-inactivating Kv conductances, but their nocturnal and less visually-guided relatives do not generally exhibit strongly inactivating Kv conductances: a positive correlation was found between the extent that a species relies on vision and a density of sustained Kv conductance within the physiological voltage range. This has raised questions if the pattern is specific to flies, or whether other insect orders evolved their own suites of electrophysiological features.

While the majority of insects possess wings, only a fraction of those flies habitually. It should therefore be interesting to test how flying affects photoreceptor function when it is not the main mode of locomotion. One way to investigate this is to compare electrophysiological properties of related species with overall similar lifestyle but different flying habits (Niven et al., 2007). We have previously characterized photoreceptors in the American cockroach Periplaneta americana (Blattidae) in detail (Heimonen et al., 2012; Heimonen et al., 2006; Immonen et al., 2014b). Periplaneta is able to fly but in laboratory conditions it flies rarely and only at high ambient temperatures. In comparison to Periplaneta, many cockroaches from families Blaberidae and Blattellidae fly more habitually. One such species is the tropical green Cuban cockroach Panchlora nivea (Blaberidae); it shares many morphological and behaviour features with Periplaneta, including nocturnal lifestyle but flies easily when disturbed even at room temperature.
This study aims at 1) describing anatomical and electrophysiological properties of photoreceptors from the compound eyes of *Panchlora* using microscopy and whole-cell patch-clamp recordings, 2) investigating the main differences between photoreceptors of *Panchlora* and *Periplaneta*, and 3) examining them in light of differing evolutionary histories and behaviours. What differences in photoreceptor properties could be expected *a priori* between *Periplaneta* and *Panchlora* if vision is assumed to be important for in-flight navigation of *Panchlora*? Based on the studies in Dipterans, *Panchlora* photoreceptors should have a relatively small membrane time constant, high density of the sustained Kv conductance, high membrane corner frequency, low membrane time constant, high density of the sustained Kv conductance, high membrane corner frequency, low membrane gain, high signal-to-noise ratio and information rate (Frolov et al., 2016; Laughlin and Weckström, 1993). As shown here, some of these predictions were not validated, suggesting a possibility of distinct evolutionary strategies for dealing with visual ecological challenges in Blattodea.

**MATERIALS AND METHODS**

All experiments described here were carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki).

**Animals**

Cuban cockroaches (*Panchlora nivea*) of both sexes were purchased from Virginia Cheeseman (Virginia Cheeseman - Entomological Supplier, High Wycombe, Buckinghamshire, UK). American cockroaches (*Periplaneta americana*, males only) were purchased from Blades Biological (Blades Biological Ltd, Edenbridge, Kent, UK), and were also given by Prof Hiroshi Nishino of Hokkaido University from his stock culture.

**Histology**

Light and electron microscopy was performed as described previously (Matsushita et al., 2012). In brief, isolated eyes were pre-fixed in 1% paraformaldehyde and 4% glutaraldehyde in 0.1 M sodium cacodylate buffer (CB; pH 7.3) for overnight at 4°C. After a brief wash with CB, the eyes were post-fixed in 2% osmium tetroxide in CB for 2 h at 20–25°C. Following *en bloc* staining with 2% UrAc in 50% ethanol, dehydration with ethanol series, and infiltration with propylene oxide, eyes were embedded in Quetol 812 (Nissin EM, Tokyo). For light microscopy (LM), 5 µm sections were examined under a light microscope (BX51, Olympus, Tokyo) equipped with a digital camera (DP71, Olympus, Tokyo). For electron microscopy (EM), ultrathin sections double-stained with uranyl acetate and lead citrate were examined with a transmission electron microscope (H7650, Hitachi, Tokyo).
For estimating volumes of ommatidium and rhabdom, we first measured the areas of individual ommatidia and rhabdoms in the 5 µm-thick serial LM sections using Image J software (NIH, Bethesda MD, USA). We then multiplied the areas by the section thickness to obtain the unit volumes, which were summed over the entire length of the ommatidium. We also measured the cross-sectional areas of ommatidia and rhabdoms in EM sections cut immediately below the crystalline cone using iTEM software (Soft Imaging System, Riverside, CA, USA), and used these values to adjust the measurement in LM sections.

**Electrophysiology**

Ommatidia were dissociated and whole-cell recordings were performed as described previously (Frolov, 2015). In brief, an Axopatch 1-D patch-clamp amplifier and pClamp software (Axon Instruments/Molecular Devices, CA, USA) were used for data acquisition. Patch electrodes were fabricated from thin-walled borosilicate glass (World Precision Instruments, Sarasota, FL, USA). Electrodes had a resistance of 5.0 – 10.0 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): 120 K-glutamate plus 20 KCl (or 140 KCl for *Periplaneta* recordings), 10 TES, 2 MgCl₂, 4 Mg-ATP, 0.4 Na-GTP and 1 NAD, pH 7.15. The differences in composition of intracellular solutions did not affect photoreceptor properties with the exception of inward hyperpolarization-activated Cl⁻ current (Salmela et al., 2012), which was suppressed in the presence of K-glutamate. The liquid junction potential (LJP) between bath and intracellular solution was −12 mV in *Panchlora* experiments and -4 mV in *Periplaneta* experiments. All voltage values cited in the text were corrected for the LJP. The series resistance was compensated by 80%. Membrane capacitance was calculated from the total charge flowing during capacitive transients for voltage steps from −112 to −92/−82 mV.

Ten monochromatic LEDs (355, 405, 435, 470, 490, 525, 535, 591, 625 and 639 nm) combined with a series of neutral density filters (ranging from 0 to 8 log units of attenuation) were used for light stimulation. To determine the spectral class of photoreceptors, a stimulus consisting of 20 ms isoquantal flashes of light from all ten LEDs was used. Recordings were performed at room temperature (21-22°C).

**Data analysis**

To evaluate photoreceptor performance, two contrast-modulated light stimuli, a naturalistic and Gaussian white-noise, were used as described previously (Frolov, 2015). The white-noise stimulus was made of 30 repetitions of a 2 s Gaussian randomly modulated sequence preceded by an adapting 1 s steady light to accommodate the initial depolarizing transient. The stimulus had a contrast of 0.36 and a 3 dB cut off (corner) frequency of 50 Hz. The analysis of responses to white-noise stimulus was performed using Matlab 7.5 (MathWorks, Natick, Massachusetts, USA), first by averaging 30 repeats in the time domain to obtain
the 2 s signal, and then subtracting the signal from each individual response to get noise traces, spectra of which were subsequently averaged. The signal-to-noise ratio (SNR) was calculated in the frequency domain and information rate was obtained according to the Shannon equation

$$IR = \int \left( \log_2 \left( \frac{|S(f)|}{|N(f)|+1} \right) \right) df$$

(Shannon, 1948) within a frequency range from 0.5 to 50.0 Hz (Frolov, 2015). In the analysis of responses to the naturalistic stimulus, the SNR was obtained from the coherence function. The contrast gain of voltage responses was calculated by dividing the cross-spectrum of photoreceptor input and output by the autospectrum of the input and taking the absolute value of the resulting frequency response function.

**Statistics**

During statistical analysis the Shapiro-Wilk normality test was first applied to data samples to determine if parametric or non-parametric statistical tests need to be used. The samples that did not pass the normality test were described with medians and interquartile ranges (25% quartile:75% quartile), and tested using the Mann-Whitney U test (MWUT). The samples that passed the normality test were analyzed with parametric statistical methods as indicated. Such data are presented as mean ± s.d. and compared using a two-tailed unpaired t-test with unequal variances. Spearman’s Rank Order Correlation Coefficient (SROCC, \(\rho\)) was used in the analysis of correlations. Spearman’s \(\rho\) was considered significantly different from zero when \(P < 0.05\). Throughout the text (\(n\)) indicates sample size.

**RESULTS**

**Anatomy**

Figure 1 shows photographs of heads of female and male *Panchlora* cockroaches. There is obvious sexual dimorphism in the head size and eye morphology. In most males the distance between eyes was very small, up to the point of confluence: 0.096 ± 0.03 mm vs. 0.272 ± 0.068 mm in females (\(n = 12\) in each sample, \(P < 10^{-3}\), t-test). This dorsal region faces forward and can be used for binocular vision. Having more facets in this region might be linked to better stereopsis in males. Since no similarly prominent sexual differences were found in *Periplaneta* (Paulus Saari, personal communications), this development may be related to flight, which *Panchlora* males undertake more readily than females, according to our observations. This however remains to be studied through detailed behavioral experiments.

Light and electron microscopy (LM and EM, respectively) were performed to study the anatomy of the *Panchlora* eye, and the differences between *Panchlora* and *Periplaneta* (Fig. 2). Both species share the apposition eye design. Although we could not detect any clear differences in the optical apparatuses (Fig. 2B, D), we saw considerable differences in the photoreceptor layer. Figure 2A shows schematic drawings of ommatidia. In both species, the ommatidium contains 8 photoreceptors (R1-8), but the arrangements of photoreceptors are dissimilar. The rhabdom of *Panchlora* is two-tiered where different sets of photoreceptor contribute in the distal and the proximal tiers, respectively. This is in contrast to *Periplaneta*,
where all photoreceptors contribute their rhabdomeres along the entire axis of the ommatidium (Butler, 1973).

We used 5 µm-thick LM sections to estimate the rhabdom length and the volume of the ommatidium (Fig. 2B-E). The average rhabdom length was 182 µm in *Periplaneta* and 114 µm in *Panchlora* (Table 1). The average ommatidial volume was 143505 µm$^3$ in *Periplaneta* but only 54063 µm$^3$ in *Panchlora*. Rhabdom volumes were 11910 µm$^3$ and 6478 µm$^3$ for *Periplaneta* and *Panchlora*, respectively (Table 1).

Electron micrographs of the retinal sections made immediately below the crystalline cone (140 µm and 75 µm from the corneal surface for *Periplaneta* and *Panchlora*, respectively) show that four large photoreceptors contribute to x-shaped rhabdoms in *Panchlora* (Fig. 2G). In contrast, rhabdoms in *Periplaneta* are characterized by irregular shapes and surrounded by much bigger pigment granules than in *Panchlora* (Fig. 2F). On average, the rhabdom cross-section area was 42 ± 16 µm$^2$ ($n = 8$) in *Periplaneta* and 81 ± 12 µm$^2$ ($n = 11$) in *Panchlora*.

In order to estimate the number of microvilli per a rhabdomere, we measured microvillar lengths and diameters using high magnification EM images. Microvillar lengths were similar in both species, 2 µm on average. Likewise, microvillar diameters were 69.8 nm in *Periplaneta* (Fig. 2H) and 68.2 nm in *Panchlora* (Fig. 2I). The numbers of microvilli per rhabdom were obtained by dividing the rhabdom volume value with the average microvillus volume value, yielding 1338400 microvilli for *Periplaneta* and 762480 for *Panchlora* (Table 1).

**Elementary responses to light**

Dissociated *Panchlora* ommatidia were notably smaller both in length and diameter, and had less screening pigment than ommatidia of *Periplaneta*. The two-tiered organization of ommatidia in *Panchlora* was often clearly identifiable by eye. Out of more than thirty *Panchlora* photoreceptors, from which light responses were obtained, all except three showed broad spectral sensitivity, responding strongest to the green LED (520 nm). The other three cells responded exclusively to UV and violet LEDs (355 and 405 nm). Since photoreceptors in cockroach ommatidia form a fused rhabdom and not an open type as in flies, all photoreceptor cells are probably physically accessible for patching, suggesting that UV-sensitive cells are present in the majority of ommatidia.

Elementary current and voltage responses of a microvillar photoreceptor to discrete photons of light are known as current and voltage quantum bumps, respectively. Voltage bumps are complex derivatives of current bump amplitude, momentary membrane resistance and membrane time constant. The latter two factors change dynamically with voltage and time, while the former is influenced powerfully by the history of photoreceptor activity.
Current quantum bumps (henceforth quantum bumps) were elicited by either a 1 ms flash of light of such intensity as to evoke single bumps with ca. 50% probability, or continuous light of low intensity, or after prolonged stimulation with bright light. The first protocol was used to study bump amplitude and latency, the second – absolute sensitivity to light, and the third – bump adaptation. The first two protocols were applied to dark-adapted photoreceptors, which were not exposed to light for over five minutes.

Figure 3A shows a typical average quantum bump recorded from a Panchlora photoreceptor at a holding potential of -82 mV. The average quantum bump amplitude of Panchlora was 27.0 ± 11.7 pA (n = 20 cells), approximately three times higher than in Drosophila (Henderson et al., 2000) but smaller than in Periplaneta, -41.1 ± 14.8 pA (n = 26). Figure 3B shows an example of latency distribution for quantum bumps recorded from one cell (the same cell as in Fig. 3A). The average latency was 50.4 ± 7.3 ms (n = 9). In comparison, bumps in Periplaneta had average latency of 62.2 ± 14.1 ms (n = 18, P < 0.01, t-test).

Absolute sensitivity to light was measured using responses to continuous low intensity light, which on average evoked ≤ 10 bumps s⁻¹. Similarly to other species (Frolov, 2016), a positive correlation was found between sensitivity and capacitance. Figure 4 shows plots of sensitivity vs. capacitance for Panchlora and Periplaneta. Spearman’s Rank Order Correlation Coefficient was 0.79 in Periplaneta (P < 10⁻⁵, n = 35) but only 0.37 in Panchlora (P = 0.041, n = 31). Note that the average capacitance of Panchlora photoreceptors was much smaller than in Periplaneta: 262 ± 72 pF (n = 56) vs. 408 ± 138 pF (n = 70), respectively. However, despite smaller size, Panchlora photoreceptors were overall as sensitive as photoreceptors in Periplaneta (Fig. 4, coinciding dotted lines indicate sensitivity medians). In Periplaneta, median absolute sensitivity was 0.40 (0.17:1.40) bumps s⁻¹ (for males only, at the same intensity as in Fig. 4). In comparison, in Panchlora, median absolute sensitivity was 0.48 (0.22:1.35) bumps s⁻¹. Our data suggest that Panchlora males have more sensitive photoreceptors than females: absolute sensitivity was 0.65 (0.40:1.92) in males (n = 15) vs. 0.33 (0.18:0.50) in females (n = 16, P = 0.01, Mann-Whitney U test). Correspondingly, capacitance values were 285 ± 107 and 261 ± 65 pF.

How do quantum bumps in Panchlora adapt to bright light? Bump adaptation is an essential mechanism that limits membrane gain and prevents excessive membrane depolarization. In practice, bump adaptation complements the voltage shunting mechanism that results from decreased driving force for light-induced current (LIC) during depolarization. To assess the bump amplitude adaptation, we compared the quantum bumps evoked in the dark-adapted photoreceptors by continuous stimulation by very dim light as described above (control) to bumps evoked in similarly dim light immediately after 60 s stimulation by progressively brighter stimuli (pre-pulses).

Figure 5A depicts averaged quantum bumps obtained in control (black trace) using a light stimulus that evoked 3.4 bumps s⁻¹ on average in this photoreceptor, and then after adapting pre-pulses in ten-fold
intensity increments. Figure 5B shows the dependence of quantum bump amplitude on pre-pulse intensity for three photoreceptors. On average, the bump amplitude decreased eight-fold from the dark-adapted photoreceptor to the recording after the brightest pre-pulse. However, these results underestimate bump amplitude reduction, since after high intensity pre-pulses only the largest bumps could be extracted from the background noise for analysis.

Macroscopic responses and information capacity

Figure 6 shows typical macroscopic voltage (Fig. 6A) and LIC (Fig. 6B) responses of Panchlora photoreceptors to a 60 s white-noise modulated stimulus over a physiological range of light intensities, from approximately 500 (black trace) to 500000 (blue trace) effective photons per second (eff. ph. s\(^{-1}\)). However, responses tended to saturate over the course of recordings in the brightest light, which manifested in a gradual decrease in depolarization/LIC amplitude, diminishing contrast resolution, and disappearance of rapid components of the response. Such saturation is likely to be due to unnatural side-on illumination in the absence of the normal optical adaptation mechanisms, and was observed in moderately bright light in virtually all photoreceptors.

Figure 6C shows voltage-light intensity relationships for peak (blue traces) and mean sustained (red traces) depolarization for the entire experimental sample (18 cells). There is a notable variability from cell to cell in the degree of depolarization. However, unlike in Periplaneta, this variability depended neither on the photoreceptor size, nor on the sensitivity to light (Immonen et al., 2014b).

Figures 6D-F show membrane gain and SNR functions, and information rate (IR) values obtained from the voltage responses shown in Fig. 6A. With increasing light intensity, shot noise decreased, while gain, SNR, and IR increased up to the level of 50000 eff. ph. s\(^{-1}\) (green trace). In still brighter light, IR decreased due to saturation. The maximal IR (IR\(_{\text{max}}\)) was on average 23 ± 9 bits s\(^{-1}\) when the entire 60 s (30 repeats of a 2 s white-noise sequence) stimulus was used for analysis. However, when only the first 10 repeats were used, the average IR\(_{\text{max}}\) was 33 ± 12 bits s\(^{-1}\), indicating a drastic decrease in information capacity for prolonged responses in moderately bright light (however, notice that the residual noise level is 1.73 times higher when 10 repetitions are used for signal estimation instead of 30). In contrast to several other species (Frolov et al., 2012; Frolov and Weckström, 2014; Frolov, 2015; Immonen et al., 2014a), no significant correlation could be found between photoreceptor capacitance, a proxy for cell size, and maximal information rate.

The membrane corner frequency was obtained by fitting the frequency-dependence of gain with a first-order Lorentzian function. For the photoreceptor responses shown in Fig. 6A the corner frequency values changed from 6 Hz at the two lowest intensities to 4.7 and 3.6 Hz at the two brightest levels. This decrease in corner frequency illustrates the effects of saturation on the relative transfer of high
frequencies.

**Potassium currents**

As in many other insect species, at least two types of voltage-activated outward currents can be electrophysiologically and pharmacologically distinguished in *Panchlora* photoreceptors (Fig. 7). Figure 7A shows the total Kv current elicited from a holding potential of −82 mV by 400 ms pulses between −82 and +28 mV in 10 mV increments, and Fig. 7B demonstrates the non-inactivating fraction of the current (IDR). The transient A-type current (IA) with hall-marks of the Shaker-like Kv current can usually be completely removed using several hundred millisecond-long inactivating voltage pulses between -50 and -40 mV (Fig. 7C). IA was measured by subtracting current traces evoked after a depolarizing pre-pulse from the currents recorded after a hyperpolarizing pre-pulse to -102 mV, which was used to recover IA channels from inactivation (Fig. 7C). This current can also be almost completely blocked by application of 2 mM 4-AP (4-aminopyridine, data not shown).

The functional availability of IA channels was tested using a steady-state inactivation protocol consisting of 500 ms pre-pulses between -112 and -12 mV followed by a testing pulse to +28 mV (Fig. 7D, E, black trace). The “window” for IA current had a peak at -50 mV indicating that IA can be involved in modulation of short depolarizing responses over most of the physiological voltage range. The half-activation potential was -34.6 ± 7.4 mV for IA and -34.5 ± 7.1 mV for IDR. As previously reported for several other insect species, larger photoreceptors expressed larger IDR than smaller photoreceptors: a moderate positive correlation was found between capacitance and average IDR in the physiological voltage range between -52 and -32 mV (ρ = 0.46, P = 0.024, n = 24, Fig. 7F). No significant correlation was detected between capacitance and IA (Fig. 7F).

In contrast to *Periplaneta*, the expression patterns of IA and IDR in *Panchlora* varied little from cell to cell. In *Periplaneta*, the majority of photoreceptors expressed a large slowly-activating IDR with a minuscule IA, while a minor fraction of photoreceptors displays a prominent IA with a smaller IDR (Salmela et al., 2012). Photoreceptors of the first kind were usually characterized by relatively high capacitance and “normal” sustained depolarization, whereas cells of the second kind were comparatively small and demonstrated low depolarization (hence dubbed “hyperadapting”). In *Panchlora*, no such variability could be detected, with all cells showing a distinct IA that usually exceeded IDR in terms of peak conductance. Out of more than 30 cells, only one showed light responses resembling the hyperadapting photoreceptors of *Periplaneta*.

**Panchlora outperforms Periplaneta**

Information transfer properties were compared using responses to a 60 s naturalistic light contrast applied over a range of light intensities from a moderately dark (eliciting less than one hundred effective photons
per second) to a saturating one (see typical voltage responses Fig. 8A). Figure 8B shows “voltage signals” obtained by averaging IR_{max} traces with the highest information rate in each photoreceptor. The *Panchlora* trace clearly demonstrates a higher gain of the light response. Figure 8C presents averaged gain functions and Fig. 8D shows SNR functions for the IR_{max} recordings. The average IR_{max} values were 25.1 ± 7.9 bits s^{-1} in *Panchlora* and 16.6 ± 6.5 bits s^{-1} in *Periplaneta* (P = 0.003, t-test) (Fig. 8E). The corner frequency of IR_{max} responses was 6.1 ± 2.3 Hz (n = 15) in *Panchlora* and 4.6 ± 1.5 Hz (n = 16) in *Periplaneta* (P = 0.042, t-test).

What are the probable causes for the superior performance of *Panchlora* photoreceptors? Firstly, the LIC of microvillar photoreceptors depends both on the number of available sampling units and intrinsic noise of the system. Microvilli are the sampling units of the photoreceptor; their functional availability is determined by the total number of microvilli, the fraction of microvilli inactivated by light, and the rate of recovery from inactivation (Song and Juusola, 2014). The higher the recovery rate, the more microvilli can be activated at any moment of time. Taking into account bump adaptation (Fig. 5), the momentary amplitude of sustained LIC should be proportional to the number of available sampling units.

Secondly, membrane filtering can in theory restrict temporal resolution of the voltage response to contrast-modulated light if sustained Kv conductance is low. Therefore, it was necessary to examine both sustained LIC and Kv currents. The maximal sustained LIC values were obtained from LIC responses to stimuli of different intensities up to a saturating level by averaging the entire duration of the current response with exception of the initial transient. Average maximal sustained LIC was notably lower in *Panchlora* than in *Periplaneta*: -136 ± 81 pA (n = 14) vs. -218 ± 135 pA (n = 10), respectively (P = 0.05, t-test, Fig. 8F). However, due to lower capacitance, average maximal sustained LIC density in *Panchlora* was slightly higher than in *Periplaneta*: -0.59 ± 0.51 pA pF^{-1} vs. -0.48 ± 0.30 pA pF^{-1}, respectively (Fig. 8G). Likewise, the sustained Kv current was much higher in *Periplaneta* than in *Panchlora* (Fig. 8J), although Kv densities in the physiological voltage range were comparable (Fig. 8H). Considering the differences in membrane capacitance, it is likely that the permissible membrane bandwidths are quite similar for both species.

However, assuming that light adaptation of quantum bumps occurs in both cockroaches to the same extent, and considering that current quantum bumps in *Panchlora* are much smaller than in *Periplaneta*, it is likely that the actual number of effective sampling units, i.e. the size of the microvillus pool available for activation by light at the intensity that produces IR_{max}, may be substantially higher in *Panchlora* than in *Periplaneta* despite the smaller maximal LIC in the former. While this can explain some of the differences in gain and temporal resolution, further examination of quantum bumps provided additional clues: current bump half-width was 25.4 ± 6.5 ms (n = 16) in *Panchlora* and 35.7 ± 2.6 ms (n = 8) in *Periplaneta* (P < 10^{-3}, t-test) (Fig. 8I), suggesting that transducer noise is lower in *Panchlora* than in *Periplaneta*. 
DISCUSSION

In this work we compared retinal morphology and electrophysiological properties of photoreceptors in two cockroach species, of which one – *Panchlora* – flies habitually and readily, while the other – *Periplaneta* – only occasionally. We tested applicability of an evolutionary visual ecological paradigm that was developed for Dipterans in 1990s (Laughlin and Weckström, 1993; Weckström and Laughlin, 1995) and recently elaborated for a more diverse range of species (Frolov et al., 2016). Accordingly, it was expected that the flying cockroach would have photoreceptors with a smaller membrane time constant, lower membrane gain, higher membrane corner frequency, higher information capacity, lower input resistance, less inactivating Kv current of higher amplitude and greater density in the physiological voltage range. Although *Panchlora* photoreceptors had higher membrane corner frequency and information capacity than photoreceptors in *Periplaneta*, other traits were not consistent with the pattern discovered in flies: *Panchlora* photoreceptors had higher membrane gain and a stronger inactivating Kv current with a smaller sustained Kv current.

The anatomical differences between *Panchlora* and *Periplaneta* retinas were striking in two aspects, the organization of ommatidia and the size of screening pigment granules. Although the latter cannot be plausibly explained by visual ecological or behavioral considerations and probably reflects a large evolutionary distance between the two species, the two-tiered rhabdom in *Panchlora* might be specifically associated with flying. Such two-tiered rhabdom usually serves to sharpen up color or polarization vision (Belusic et al., 2017), and while *Panchlora* has only green- and UV-sensitive photoreceptors, meaning no proper color vision, *Panchlora* might use polarized light for navigation during flight in the dark.

The first question that arises in regard to relationships between visual ecology and photoreceptor biophysics is whether differences in electrophysiological properties of photoreceptors are related to behavior at all, considering that the original observations in *Periplaneta* itself were anything but consistent with the above-mentioned visual ecological theory. Could it be that different insect orders evolved dissimilar suites of visual ecological adaptations? Although proper investigation of such general questions must rely on thorough comparative studies, evidence available at this stage already indicates that cockroaches might indeed have used independent methods to deal with evolutionary challenges to visual systems. The Kv has been recorded from two more cockroach species, a large wingless nocturnal crawler, *Gromphadorhina portentosa*, (Blaberidae), also known as the Madagascar hissing cockroach, and the crepuscular flying “dusky” Lapland cockroach *Ectobius lapponicus* (Blattellidae) (Frolov et al., 2016). There were notable similarities between *Periplaneta* and *Gromphadorhina*, and between *Panchlora* and *Ectobius*. Like *Periplaneta, Gromphadorhina* photoreceptors are large and express a prominent delayed rectifier with relatively little inactivation. IA current is comparatively small in both species (Frolov et al., 2016). In
contrast, photoreceptors in the miniature *Ectobius* appear to be smaller than in *Panchlora*, and display a very similar Kv current with a dominant IA and relatively small IDR.

There is circumstantial evidence that these results are not a coincidence but an evolutionary trend. *Periplaneta* demonstrates large morphological and physiological variability in all aspects of organization of its visual system periphery (Butler, 1973; Heimonen, 2008; Heimonen et al., 2006; Ribi, 1977). This variation is not ordered, unlike, for instance, the variation in photoreceptor sizes in the ommatidia of the backswimmer *Notonecta glauca* (Immonen et al., 2014a). Rather, it is intrinsically random, and can be a sign of primitive organization. Variability in *Periplaneta* photoreceptor size, absolute sensitivity to light, voltage response waveforms, amplitude and kinetics of light-induced and Kv currents are prominent (Heimonen et al., 2006; Salmela et al., 2012). In contrast, *Panchlora* photoreceptors demonstrated a comparatively low variability in electrophysiological properties with high functional efficiency. Superior efficiency manifested in improved information processing (see below) and in median absolute sensitivity that matched *Periplaneta*'s despite the smaller membrane area of photoreceptors as it follows from comparison of average capacitance values (1.6-fold difference) or rhabdom volume estimates (1.8-fold difference) (Table 1). However, more morphological studies are needed to establish if stochastic variability in visual systems of cockroaches diminishes with increased specialization and natural selection pressure on visual performance.

If increased inactivation of Kv conductance is indeed a feature of more sophisticated Blattodea visual systems, then how can it be reconciled with the observations in Diptera? First, the length of photoreceptor axons differs between cockroaches and flies: in *Periplaneta*, axon lengths vary between 300 and 1500 μm (Heimonen et al., 2006), while in flies axons are much shorter, around 100 μm in *Drosophila* for R1-6 photoreceptors (Pollock et al., 1990). This indicates that graded signals transferred along the axon attenuate much more strongly in the cockroach, and that action potentials (APs) sporadically recorded in cockroach photoreceptors (Heimonen et al., 2006) may reduce information loss by regenerative amplification of higher frequencies in the response. Expression of a transient Kv conductance (Kv1, Shaker) in the axon initial segment is crucial for regulation of sub-threshold membrane potentials, AP initiation and the shape of the propagating APs (Clark et al., 2009). Also, by reducing the length constant in a voltage- and time-dependent manner, increased expression of IA can facilitate high-pass filtering of propagated graded signals (Rusanen and Weckström, 2016). Thus it is possible that flying cockroaches such as *Panchlora* and *Ectobius* need higher IA conductance than the crawlers like *Periplaneta* and *Gromphadorhina*. Alternatively, non-spiking photoreceptors of dipterans (Fuortes and O’Bryan, 1972) may need transient Kv conductances for a different purpose, unrelated to signal transfer along the axon, possibly to avoid unnecessary metabolic expenses associated with large sustained Kv conductances (Niven and Laughlin, 2008).
It was shown here that photoreceptors of *Panchlora* have higher information rates than photoreceptors of *Periplaneta*, due to increased gain and membrane corner frequency. What could explain these differences? We hypothesize that one cause might be the differences in transducer noise that apparently favor *Panchlora* over *Periplaneta*. There are three sources of intrinsic noise in microvillar photoreceptors: spontaneous activation of phototransduction cascade, thermal noise, and transducer noise (Laughlin and Lillywhite, 1982; Lillywhite and Laughlin, 1979). However, contributions of the first two kinds are negligible in comparison with the transducer noise. Transducer noise has three components, which are variations in bump shape ("bump shape noise"), latency ("latency noise"), and amplitude ("shot noise"). Statistical analysis of quantum bumps in the cockroach (Immonen et al., 2014b) implies that all three components of the transducer noise are mutually independent and intrinsically stochastic. Unlike the bump shape and latency noises, shot noise is largely inconsequential for information transfer by the membrane because the effect of differing amplitudes of individual bumps constituting an aggregated response to light will be canceled out by averaging, except perhaps in very dim light when single bumps dominate the response. Indeed, it was suggested that shot noise may not affect information capacity at all (Salmela, 2013).

Noise caused by variations in bump latency and shape, however, can affect the kinetics and amplitude of the aggregated macroscopic response. Latency noise adds stochastic desynchronization of bump onset times, leading to a broader response of smaller amplitude than in the absence of such variation, an effect similar to low-pass filtering. Importantly, since latency is considered to be a Poisson process, latency variation equals mean, with an apparent consequence that in order to improve synchronization of bump summation, mean latency need be shortened. This is generally consistent with a visual ecological pattern observed among insects: the species that rely heavily on vision have shorter photoreceptor response latencies than less visually dependent organisms. Also, since response latency decreases strongly with increasing brightness of the stimulus (about two-fold in *Panchlora* from the background intensity “-4” to “-1” as in Fig. 6C), latency noise should also diminish, with concomitant increase in gain, temporal resolution and SNR. Comparison of mean latencies of *Panchlora* (50.4 ms) and *Periplaneta* (62.2 ms) photoreceptors implies that latency noise should indeed be relatively small in *Panchlora*. Likewise, the bump shape noise is expected to increase the duration and decrease the amplitude of a macroscopic response thus reducing gain and membrane corner frequency. Although the relative contributions of latency and bump shape noises are not known, both are significantly smaller in *Panchlora* than in *Periplaneta*.

One of the shortcomings of this work is that no attempt was made to evaluate possible regional differences in photoreceptor function, which might stem from the well-known regional morphological specializations such as the genuine specialized areas (e.g. the dorsal rim area in crickets (Frolov et al.,
2014)) or various high-acuity zones (Land, 1997). Do photoreceptors in such high acuity regions process information better, i.e. faster and with higher SNR, than in the rest of the eye as would be warranted by visual ecological considerations? However, neither Periplaneta, nor Panchlora possess any morphologically distinct regions. The present electrophysiological method, patch-clamp recordings from photoreceptors in dissociated ommatidia, is particularly unsuited for addressing this question, requiring the intracellular recordings approach. Nevertheless, we can safely assume that any putative variation in photoreceptor performance due to regional differences falls within the variabilities in electrophysiological properties described here.

In conclusion, this work described the biophysical properties of photoreceptors in the cockroach Panchlora nivea, and is the second detailed study of a Blattodea species. The findings raise the possibility that due to different morphologies of visual systems, and, probably, different roles of visual sense in physiology and behavior, evolutionary adjustment of photoreceptor properties to visually-demanding behaviors proceeded in the comparatively “non-visual” Blattodea along different lines than in the highly visual Diptera, a hypothesis that needs to be tested in further comparative studies.
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Competing interests

No competing interests declared.

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References


Fig. 1. Sexual dimorphism in *Panchlora* eyes.
Fig. 2. Retinal anatomy in Periplaneta americana and Panchlora nivea. (A) Schematic drawing of ommatidium of Periplaneta (P.a) and Panchlora (P.n.). Upper pictures show transverse sectional view of the distal layer of the ommatidium. (B, C) Longitudinal and transverse (immediately below the crystalline cone) sections of the middle region of a Periplaneta eye. (D, E) Same as B and C for Panchlora. Rhabdoms (arrowheads) are densely surrounded by pigments. Areas of ommatidia and rhabdoms were obtained by tracing their contours, and used for estimating volumes. (F, G) Transmission electron micrographs of
transverse sections of light-adapted rhabdoms (R) of *Periplaneta* (F) and *Panchlora* (G). The arrow in F indicates proximal tip of a crystalline cone, which is surrounded by rhabdomeres. Insets: Microvillar base, showing pigment granules (P). (H, I) Cross sectioned microvilli of *Periplaneta* (H) and *Panchlora* (I). Arrowheads show lipid bilayers.
Fig. 3. Quantum bumps. (A) A typical average quantum bump of *Panchlora* photoreceptors was obtained by averaging bumps elicited with 1 ms flashes of light (arrow) from a holding potential of -82 mV. (B) Quantum bump latency distribution is shown for the same photoreceptor. Latency values were obtained by measuring the intervals between the time of flash and the time when bump amplitude reached 2 pA.
Fig. 4. **Absolute sensitivity and capacitance.** The sensitivity values were obtained by counting quantum bumps evoked in dark-adapted photoreceptors in continuous dim light of such intensity as to evoke less than 10 bumps per second; bump rates were recalculated for the common level corresponding to the light intensity 10-fold lower than “-5” attenuation level in Fig. 6C. Vertical and horizontal lines denote capacitance and sensitivity medians (grey, *Panchlora*; black, *Periplaneta*); sensitivity medians coincide.
Fig. 5. Light adaptation of quantum bumps. (A) Average quantum bumps recorded from a dark-adapted photoreceptor with a prolonged dim light stimulus evoking 3.4 eff. ph. s\(^{-1}\) (black trace), or after a pre-pulse of incrementing intensity; intensity of the pre-pulse is presented in terms of eff. ph. s\(^{-1}\). (B) Dependence of average quantum bump amplitude on pre-pulse intensity for three photoreceptors (white, grey and black symbols); a fitted trace is a common trendline.
Fig. 6. Macroscopic responses and information transfer. (A) Typical voltage and (B) light-induced current (LIC) responses of a green-sensitive photoreceptor to white-noise modulated stimuli of different intensities; data from the same cell are shown; white-noise stimulus is shown in grey in panel A. (C) Dependence of the transient (blue traces) and steady-state (red traces) depolarization on light intensity for individual responses (lines) and the sample averages (circles); steady-state depolarization was determined as the difference between resting potential and the average plateau potential between 3 and 61 s after the onset of light. (D) Light-voltage gains (mV per unit of contrast) and (E) SNRs for responses from panel A; color coding as in panel B. (F) Dependence of information rate on stimulus intensity for the same photoreceptor.
Fig. 7. Kv conductances in *Panchlora*. Examples of the (A) total Kv current, (B) delayed rectifier IDR and (C) transient IA. IA and IDR were separated using the protocol shown in the inset of panel B, with letters denoting the panels presenting the corresponding current responses. Currents were elicited by 400 ms pulses between −82 and +28 mV in 10 mV increments from a HP of −82 mV; each testing step was preceded by a 1 s pre-pulse to either −102 mV to fully recover IA, or to −47 mV to fully inactivate IA; IA was then obtained by digital subtraction of the resultant traces (C); the first 3 ms of the current traces containing capacitive transients were removed. (D) Voltage-dependences of the average maximal Kv conductances and functional availability of IA channels; IDR was obtained at the end of 400 ms long total Kv current traces; IA availability was determined using a steady-state inactivation protocol consisting of 500 ms pre-pulses between -112 and -12 mV followed by a testing pulse to +28 mV (inset in panel E); sample sizes are provided in parentheses; error bars denote s.d. (E) Normalized voltage-dependencies from panel D. (F) Correlation between capacitance and average IDR in the physiological voltage range between -52 and -32 mV.
Fig. 8. Comparison of photoreceptor performance in *Panchlora* and *Periplaneta*. (A) Examples of voltage responses of a *Panchlora* photoreceptor to a 60 s naturalistic light contrast (grey trace) in 10-fold intensity increments; first 25 s are shown. The blue trace corresponds to the maximal information rate. (B) Averaged responses with the highest IR for both species; sample sizes are provided in parentheses. (C) Average gain (mV per unit of contrast) and (D) SNR functions for responses in panel B; throughout this figure, error bars denote s.d. (E-G) Comparison of (E) IR$_{\text{max}}$, (F) maximal sustained LIC, and (G) maximal sustained LIC density values. (H) Comparison of voltage-dependences of the average sustained Kv conductances in *Panchlora* and *Periplaneta*, and (H) their densities; red rectangle indicates the putative physiological voltage range. (I) Comparison of the sample-average quantum bumps obtained by averaging the average bumps from each photoreceptor.
Table 1. Photoreceptor properties of *Periplaneta* and *Panchlora*

<table>
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<tr>
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<th>Periplaneta</th>
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<th>Panchlora</th>
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<tr>
<td></td>
<td>mean ± SD</td>
<td>n</td>
<td>mean ± SD</td>
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<tr>
<td><strong>Anatomical properties</strong></td>
<td></td>
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<tr>
<td>Rhabdom length (µm)</td>
<td>182 ± 10</td>
<td>38</td>
<td>114 ± 8</td>
<td>30</td>
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<tr>
<td>Ommatidium volume (µm³)</td>
<td>143,505 ± 6,913</td>
<td>38</td>
<td>54,063.2 ± 2,448</td>
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<td>Rhabdom volume (µm³)</td>
<td>11,910 ± 874</td>
<td>38</td>
<td>6478 ± 30</td>
<td>30</td>
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<td>Rhabdom occupation ratio (b/a, %)</td>
<td>8.3</td>
<td></td>
<td>12.0</td>
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<td>Microvillus diameter (nm)</td>
<td>69.8 ± 6.5</td>
<td>189</td>
<td>68.2 ± 5.0</td>
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<tr>
<td>Number of microvilli in a rhabdom</td>
<td>1,338,400</td>
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<td>762,480</td>
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<td><strong>Main electrophysiological properties</strong></td>
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<tr>
<td>Bump amplitude (pA)</td>
<td>-41.1 ± 14.8</td>
<td>26^a</td>
<td>27.0 ± 11.7</td>
<td>20</td>
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<td>Bump latency (msec)</td>
<td>62.2 ± 14.1</td>
<td>9</td>
<td>50.4 ± 7.3</td>
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<tr>
<td>Bump half-width (msec)</td>
<td>35.7 ± 2.6</td>
<td>8</td>
<td>25.4 ± 6.5</td>
<td>16</td>
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<td>Photoreceptor capacitance (pF)</td>
<td>408 ± 138</td>
<td>70</td>
<td>262 ± 72 pF</td>
<td>56</td>
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<tr>
<td>Absolute sensitivity (bumps s⁻¹, medians, see Results for details)</td>
<td>0.40^d</td>
<td>35</td>
<td>0.48 (0.65°/0.33°)</td>
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<td>Corner frequency (Hz)</td>
<td>4.6 ± 1.5</td>
<td>16</td>
<td>6.1 ± 2.3</td>
<td>15</td>
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<tr>
<td>Light induced current (pA)</td>
<td>-218 ± 135</td>
<td>10</td>
<td>-136 ± 81</td>
<td>14</td>
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^a Data from the previously used control sample (Saari et al., 2017).