Photoreceptor spectral sensitivity of the compound eyes of black soldier fly (Hermetia illucens) informing the design of LED-based illumination to enhance indoor reproduction

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A B S T R A C T

Mating in the black soldier fly (BSF) is a visually mediated behaviour that under natural conditions occurs in full sunlight. Artificial light conditions promoting mating by BSF were designed based on the spectral characteristics of the compound eye retina. Electrophysiological measurements revealed that BSF ommatidia contained UV-, blue- and green-sensitive photoreceptor cells, allowing trichromatic vision. An illumination system for indoor breeding based on UV, blue and green LEDs was designed and its efficiency was compared with illumination by fluorescent tubes which have been successfully used to sustain a BSF colony for five years. Illumination by LEDs and the fluorescent tubes yielded equal numbers of egg clutches, however, the LED illumination resulted in significantly more larvae. The possibilities to optimize the current LED illumination system to better approximate the skylight illuminant and potentially optimize the larval yield are discussed.

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1. Introduction

The black soldier fly (Hermetia illucens (L.); Diptera: Stratiomyidae; BSF) is a tropical fly species with great potential for the processing of several types of organic waste and byproducts (Larde; BSF) is a tropical fly species with great potential for the processing of several types of organic waste and byproducts (Larde, 1990; Oonincx et al., 2015a,b; St-Hilaire et al., 2007). Their larvae and prepupae are rich in protein and fat, and can serve as feed for a wide range of animals, such as fish, pigs, poultry, as well as dogs and cats (Bosch et al., 2014; Makkar et al., 2014). In order to successfully breed the BSF, mass rearing protocols need to be developed (Pastor et al., 2015). Several studies report on temperature and humidity requirements of larvae and adults, as well as on conditions for pupation (Holmes et al., 2012, 2013; Sheppard et al., 2002; Tomberlin et al., 2009; Tomberlin and Sheppard, 2002). Providing these circumstances in production facilities is feasible, even in temperate regions. However, one bottleneck for the mass production of BSF is the efficient production of eggs (Pastor et al., 2015). The BSF depends on direct sunlight for mating and therefore adult colonies are normally kept in greenhouses with considerable costs of climate control (Sheppard et al., 2002; Tomberlin and Sheppard, 2002; Zhang et al., 2010).

Some attempts have been made to induce mating under artificial conditions. Zhang et al. (2010) tested a so-called rare earth-lamp (500 W, emitted wavelengths between 350 and 450 nm) which failed to stimulate mating. However, a quartz-iodine lamp (450 W, emitted wavelengths between 350 and 2500 nm), did induce mating (Zhang et al., 2010). The spectrum of the latter lamp supposedly was similar to sunlight. Because the rare earth lamp was ineffective, the researchers concluded that wavelengths between 450 and 700 nm influence mating behaviour. Similarly, Tomberlin and Sheppard (2002) tried two artificial light sources (a 40-w Sylvania Gro Lux® and a 430-w ProUltralight Light System®), but did not observe mating, and obtained only infertile eggs. When their colony was provided with direct sunlight through a window, mating was observed and fertilized eggs were obtained. As 75% of all matings took place when the light intensity was above 200 μmol m⁻² s⁻¹ and adult BSFs are active on sunny days only, Tomberlin and Sheppard (2002) concluded that intense sunlight was a major determining factor for mating, and they suggested that to induce mating male BSF need specific wavelengths which were absent in the artificial light sources.

In diurnal insects, the visually mediated behaviour is supported by the detection of shapes, colours, patterns or movement by the visual system (Engelmann and Kerkut, 1970). The visual system of adult BSF consists of three simple eyes (ocelli) and a pair of large compound eyes. In many insect species mating involves the
airborne interception of females (Horridge and Mclean, 1978; Ruttner and Ruttner, 1965; Vogel, 1957). Similarly, the BSF males exhibit “lekking behaviour”, i.e. they sit on leaves and intercept flying conspecifics. If a male is intercepted, a fight ensues; if a female is intercepted, copulation is initiated in flight (Tomberlin and Sheppard, 2001). From below, the bodies of conspecifics are perceived as dark spots against the sky. The contrast between the sky and the insect silhouette is strongest in the ultraviolet part of the light spectrum (Belušić et al., 2013). The dorsal retina of many insects predominantly contains blue- or ultraviolet-sensitive photoreceptors (Stavenga, 1992; Stavenga et al., 2001). Insect behaviour, including mating, can be influenced by UV light (Hunt et al., 2001; Kemp, 2008; Obara et al., 2008; Shimoda and Honda, 2013).

Efforts to breed BSF indoors have to be based on an understanding of the physiological properties of the BSF visual system. The BSF belongs to a suborder of Diptera, the Brachycera. All studied species belonging to this suborder share a common design of the retina (Boschek, 1971; Hardie et al., 1989; Kuiper, 1962; Trujillo and Melamed, 1966). Each ommatidium of their compound eye contains six large cells (R1-6) of which the photosensitive parts, the rhabdomeres, are positioned at the periphery. The rhabdomeres of two smaller cells (R7, R8) reside in the centre of the ommatidium, R7 situated distally and R8 proximally. The cells R1-6 represent a spectrally homogeneous class of photoreceptors with a broad spectral sensitivity. Their fast temporal responses presumably are used for motion detection, while their broad spectral sensitivity is less suitable for colour discrimination (Gao et al., 2008; Heisenberg and Buchner, 1977; Yamaguchi et al., 2010), but at least in Drasophila, R1-6 do contribute to colour vision (Schnaitmann et al., 2013). The central photoreceptors R7 and R8 have narrow spectral sensitivities and their temporal responses are slower (Autrum et al., 1985). Photoreceptors R7 (subclasses R7y and R7p) have peak sensitivity in the UV, while the two subclasses of R8 are most sensitive to blue (R8p) or green (R8y) light. Thus, they enable trichromatic vision, based on the three channels, UV – blue – green (Gao et al., 2008; Heisenberg and Buchner, 1977; Yamaguchi et al., 2010). A similar division might be expected in the retina of the BSF, with motion and wavelength detection photoreceptor classes. In some Diptera, particularly in the true flies, the spectral sensitivity of the photoreceptors is modified by additional chromophores bound to the outer side of visual pigment molecules, the opsins. These act as sensitizing pigments, increase the UV-sensitivity and contribute a detailed spectral signature to the spectral sensitivity, the ‘fine vibronic structure’ with three sharp peaks of sensitivity between 330 and 370 nm (Stavenga et al., 1989). The sensitizing pigments have not been reported so far in the family Stratiomyidae.

Small animals such as insects cannot afford neural redundancy and their senses and brains are narrowly tuned to their natural environment. A low mating rate of BSF indoors is likely due to the indoor illumination failing to produce the necessary natural visual cues. It is crucial to understand the physiological properties of the BSF visual system in order to create the correct light circumstances that induce indoor mating in order to produce fertilized eggs. Therefore, the aims of this study were to determine the spectral sensitivity of BSF compound eyes and subsequently, design an illumination system with the appropriate wavelengths using LED technology. Illumination by LEDs and the conventional fluorescent artificial light sources were compared to the natural illuminant (skylight) in terms of the relative photoreceptor excitation. Lastly, two artificial illumination systems were provided to BSF adults to compare their effectiveness in obtaining fertilized BSF eggs.

2. Materials and methods

Black soldier flies were obtained from the breeding colony at the Laboratory of Entomology (Wageningen University, Wageningen, The Netherlands). This colony was maintained for over five years in a climate chamber at a temperature of 26 °C and a relative humidity of 70% illuminated by an array of fluorescent tubes (TLD18W840NG, Philips, Eindhoven, The Netherlands) resulting in a light intensity of approximately 23 W m⁻². Containers with pupae from this colony were kept in the dark and were checked daily for newly eclosed adults. Gender was visually determined based on genitalia (Fig. 1).
Two experiments were conducted; in the first experiment it was determined which wavelengths were detected by BSF adults, in the second experiment LEDs emitting light with those wavelengths were provided to BSF adults and the number of clutches and the number of resulting offspring was determined.

Experiment 1: Electrophysiological recordings were performed in 24 female and 20 male BSF. The results from all 44 specimens were pooled because no gender differences were identified. Prior to recordings, the legs of the BSFs were removed and the body was placed in a plastic tube with a mix of bee wax and resin to immobilize it. Sharp borosilicate microelectrodes with resistance 60–100 MΩ were prepared on a P-97 puller (Sutter Instrument Company, Novato, USA) and filled with 3 M KCl. The electrode, mounted on a piezo micromanipulator (Sensapex, Oulu, Finland), was inserted into the retina through a hole in the cornea, which was sealed with Vaseline. The reference electrode was an Ag/AgCl wire positioned in the non-illuminated eye. The animal and the micromanipulator were positioned on a fully rotatable goniometric stage with an additional x-y-z micrometer stage. The optical axis of the recorded ommatidium was aligned with the stimulating light stage with an additional x-y-z micrometer stage. The optical axis of the recorded ommatidium was aligned with the stimulating light beam to yield a maximal signal. This signal was amplified with a SEC-10 amplifier (NPI, Tamm, Germany), low-pass filtered at the Nyquist frequency with a CyberAmp 320 signal conditioner (Molecular Devices, USA), digitized with a Micro 1401 lab interface (CED, Cambridge, UK) and recorded with WinWCP software (John Dempster, University of Strathclyde, UK). A 150 W XBO Xenon arc lamp (Osram, Germany), shone through a monochromator (B&M Optik, Germany; bandpass FWHM ~ 5 nm), provided monochromatic light flashes between 255 and 760 nm in 5 nm steps for spectral sensitivity measurements. Light intensity was adjusted with a computer controlled, rotating reflective neutral density wedge with a linear gradient of attenuation between 0 and 10^-4 (Tholars, Germany). Spectral sensitivity was measured using isosquintal stimuli between 300 and 700 nm. The relationship between stimulus intensity and the voltage response of the photoreceptors was measured by light pulses at a constant wavelength with graded intensity from 10^-4 to full light in 0.5 log unit steps. These data were fitted in Prism 6.0 (Graphpad, USA) with a sigmoidal function with variable slope. The spectral sensitivities were then calculated by converting the wavelength-dependent receptor voltages into the effective light intensities using a reverse transformation of the sigmoid function. Five stable photoreceptor cells were subsequently injected with intracellular dye Alexa Fluor 568 hydrazide (Life Technologies, USA) and observed in vivo with water immersion under an epifluorescent microscope Olympus BX51 (Olympus, Japan), fitted with a 20× LWD water immersion objective (Olympus, Japan).

Black soldier fly eyes were imaged with a USB digital microscope Dino-Lite Edge AM4515ZT (AnMo Electronics corporation, Taiwan). For transmission electron microscopy, isolated eyes were fixed, contrasted and embedded with standard procedures (fixed for 3 h in 4% paraformaldehyde and 3.5% glutaraldehyde, dehydrated in ethanol (50–100% in 10% steps), incubated 90 min in 0.1 M OsO4 in 0.1 M Na-cacodylate, pH 7.4, embedded in Spurr resin). Ultrathin sections were cut with a diamond knife and observed with a Philips CM10 electron microscope.

Experiment 2: Ten male and ten female adults, kept without light prior to the experiment, were randomly allocated to a cage for testing the effect of illumination on the number of produced clutches and the number of larvae stemming from these clutches. During the experiment, flies were housed in a Bugdorm cage (30 × 30 × 30 L × W × H; type DP-1000, BugDorm store, Taiwan), at a temperature of 26 °C and a relative humidity of 70%. Sugar water (344 g sucrose/L) was provided by means of a glass bottle (30 ml) in which a folded piece of paper tissue was placed to prevent the flies from drowning. The sugar water was continuously available and refreshed twice a week. Plastic oviposition containers (15.5 × 7.5 × 6.5 cm; L × W × H) were filled for 2/3 with moist, unfertilized coco peat, on which a few grams of commercial chicken feed (Masters diervoeders, Lienden, The Netherlands) were sprinkled. These were prepared 24 h before they were put in a cage and kept in the dark at 26 °C with a relative humidity of 65%, in order to facilitate bacterial growth, which is attractive to female black soldier flies (Booth and Sheppard, 1984; Yu et al., 2011; Zheng et al., 2013). Each container had two vertically placed cardboard strips of which the top was accessible for females to oviposit into. These containers were replaced daily, and the number of egg clutches per container was counted. Furthermore, per cage the mortality for both males and females was recorded daily. After collection, oviposition containers were kept in the dark at 26 °C with a relative humidity of 65% and provided with moistened chicken feed for two weeks. These containers were checked twice a week for the presence of larvae and when needed more moistened chicken feed was added. After these two weeks the number of larvae was counted per container. All cages were illuminated for 12 h per day (8:00–20:00 h). Two light sources were used; the first were fluorescent tubes (TLD18W840NG, Philips, Eindhoven, The Netherlands), which previously had been shown to be effective in inducing mating and oviposition of fertilized eggs in our colony and are hence named control. The tube lighting was powered by high-frequency drivers HFR 236 and HFR258 (Philips, The Netherlands), operating at 45 kHz. The second light source consisted of LEDs emitting light of 365 nm, 450 nm and 515 nm (equal numbers of H2A1-H365-E, H2A1-H450 and H2A1-H515, Roithner LaserTechnik, Austria), powered with DC power supplies PTDC/10/350 (Roithner LaserTechnik, Austria). Furthermore, two light intensities were tested for each of the two light sources. Light intensity was determined by means of a radiometrically calibrated Flame spectrophotometer (Ocean Optics, USA) and a power meter (Newport, USA). The high intensity control group was kept under the same conditions as the rearing colony; placed underneath an array of tube lights at a distance of 5 mm from the top of the cage. The low intensity control group was placed underneath two tube lights at a distance of 20 mm. In the LED treatments different light intensities were provided by using either four or eight triplets (UV, blue and green) of 1 W LED’s.

The irradiance spectra of illumination systems were measured with a radiometrically calibrated Flame spectrophotometer (Ocean Optics, USA). Irradiance spectra were measured with a quartz optical fiber probe, equipped with a cosine corrector (Ocean Optics, USA), pointing into a MgO reflectance standard. The spectral distribution of the light emitted by the LEDs and by the tube lights was compared to the standard daylight illuminant CIE D65. Each treatment was first performed in duplo and this was repeated for three consecutive rounds resulting in a total of six replicates (n = 6) per treatment. For each replicate twenty newly eclosed flies (10 male and 10 female) were used, resulting in 480 flies being used in the experiment. The data did not have equal variances (Levene’s test; F = 3.27, P = 0.043), and was not normally distributed (Shapiro–Wilks test; W = 0.632, P < 0.001). Therefore it was tested for significant differences (P < 0.05) by means of a Kruskall-Wallis test followed by a Scheffé test. The Kaplan-Meier method was used to analyze longevity and a Mantel-Cox test was used to detect differences between treatments, with a Bonferroni’s correction for multiple comparisons. Statistical analysis for all data was performed using SPSS 22.0.
3. Results

3.1. Spectral sensitivity of BSF compound eyes

The retinal substrate for wavelength discrimination of BSF was first studied with microelectrode recordings. Spectral sensitivity of retinal photoreceptors was measured by recording from single cells in the dorsal, equatorial and ventral retina (Fig. 2A, regions marked with coloured dots). The cells belonging to the major photoreceptor class R1-6 (Fig. 2B, R1-6) were identified by their frequency of occurrence, their electrical and spectral properties and with intracellular dye injection (Fig. 2C). In distinct eye parts, these were the cells that were repeatedly impaled during the electrode excursion. Their receptor potentials were smooth, with low amplitude of quantum shot noise at weak flashes; strongest light flashes showed fast light adaptation (Fig. 2D). Their spectral sensitivity was broad (Fig. 2F). In the ventral retina, R1-6 photoreceptors were maximally sensitive in the UV (367 nm) and blue (440 nm) (Fig. 2F, violet curve). The high UV sensitivity was due to the presence of a sensitizing (also called antenna) pigment, as revealed by the fine vibronic structure in the spectral sensitivity recorded with 1 nm resolution (300–400 nm; Fig. 2F, black curve). At the eye equator, the R1-6 were blue-sensitive (λ\(\text{max} = 437 \pm 1\) nm; Fig. 2A, blue curve), i.e. their sensitivity in the blue part coincided with the sensitivity of the cells in the ventral part. Their sensitivity in the UV was low and lacked the fine vibronic signature of the sensitizing pigment. In the dorsal retina, most of R1-6 were maximally sensitive in the blue-green (504 nm) with an additional UV peak up to 40% of the 500 nm peak (Fig. 2F, green curve). Similar to the ventral retina, approximately 20% of R1-6 in the dorsal retina had peak sensitivity in the UV-blue. The precise photoreceptor spectral sensitivities, localization and its possible correlation with the corneal stripes (Fig. 2A) will be treated separately in another article. The R7 (Fig. 2B, R7) and R8 cells were identified by the frequency of occurrence, electrical and spectral characteristics. They were impaled very rarely (<10% of all cells). At low flash intensities, they featured high amplitude quantum shot noise; at high intensities, the receptor potentials revealed somewhat slower light adaptation than that in R1-6 (Fig. 2E). Their spectral sensitivity was narrow (Fig. 2G). These cells had sensitivity maxima in the ultraviolet at 332 and 351 nm and in the green at 535 nm. The R7 and R8 cells were identified according to the fly nomenclature (Autrum et al., 1985) as R7p (λ\(\text{max} = 332 \text{ nm}\)), R7y (λ\(\text{max} = 351 \text{ nm}\)) and R8y (λ\(\text{max} = 535 \text{ nm}\)).

3.2. Evaluation of the illumination system

The intracellularly measured photoreceptor spectral sensitivities allowed us to evaluate the effect of different illuminants on the BSF retinal photoreceptors. The irradiance spectra of artificial illumination sources were measured with a spectroradiometer to analyze their output in distinct spectral bands. For comparison, the standard daylight illuminant spectrum (CIE D65) was included into the analysis. Relative photoreceptor excitation was calculated for different illumination conditions.

The irradiance spectrum of CIE D65 is continuous and the intensity steadily increases from UV towards the long wavelengths. Both the LEDs and tubes produce discontinuous irradiance spectra between 300 and 650 nm (Fig. 3A). The irradiance spectrum of LED lighting contains three peaks with unequal amplitudes due to different efficiencies of the three LED types. The tubes’ spectrum contains several discrete, sharp irradiance peaks, including a small peak in the UV. All light sources, however, stimulate photoreceptors with broad sensitivities (bandwidth of fly photoreceptors

![Fig. 2. Retinal anatomy, regionalization and spectral sensitivity of the black soldier fly (Hermetia illucens; BSF).](image-url)

A. Head of a female BSF with the dots marking the dorsal, equatorial and ventral regions of the retina. The colours of the dots indicate the spectral sensitivity peaks of the R1-6 photoreceptors in each region: green dorsally, blue at the equator, UV-blue ventrally. B. Electron micrograph of a cross section of a BSF ommatidium, revealing an open rhabdom arrangement. The enumerated rhabdomeres mark the “outer” (1–6) and the “inner” photoreceptors (at this level, only R7 is visible; R8 is located proximally). C. Rhabdomere tips, visualized through the optically neutralized cornea with a water immersion objective and UV elicited epifluorescence. The red fluorescing, green-sensitive photoreceptor R3 in the central ommatidium has been injected with Alexa 568 dye. D, E. Responses of an R1-6 (D) and an R7 photoreceptor (E) to graded UV pulses (horizontal bar at the bottom). F. Spectral sensitivity of R1-6 photoreceptors, recorded with 1 nm resolution, from the dorsal (green curve, λ\(\text{max} = 504 \pm 1\) nm; N = 11), equatorial (blue curve, λ\(\text{max} = 437 \pm 1\) nm; N = 6) and ventral (violet curve, λ\(\text{max} = 367 \text{ nm}\); λ\(\text{max} = 440 \pm 1\) nm; N = 14) retina. Black curve, spectral sensitivity of R1-6 in the ventral retina, recorded between 300 and 400 nm with 1 nm resolution. G. Sensitivity of central photoreceptors R7 and R8 (pink curve, λ\(\text{max} = 351 \text{ nm}\), N = 6; green curve, λ\(\text{max} = 535 \text{ nm}\), N = 4; black curve, λ\(\text{max} = 332 \text{ nm}\); N = 2). The curves in F and G represent mean values from retinæ of both sexes (M, N = 20; F, N = 24); error bars indicate SEM. For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
R1-6 is typically >200 nm; that of UV- and green-sensitive R7 and R8 photoreceptors >100 nm. Therefore, the irradiance spectra were integrated into spectral bands, UV (300–400 nm), blue (400–500 nm) and long-wavelength (500–650), and the fractional irradiance \( f_{IR} \) was calculated for each spectral band (\( f_{IR_{UV, B, LW}} = \frac{I_{UV, B, LW}}{I_{UV} + I_{RB} + I_{RLW}} \) (Fig. 3B). The UV content of LEDs approximates CIE D65 quite well (\( f_{UV, LED} = 0.09; f_{UV, CIE65} = 0.10 \)), but the LED spectrum contains 2.4 times more blue light and only 0.22 of long-wavelength light compared to the CIE D65 spectrum. The irradiance spectrum of fluorescent tubes contains a small and narrow UV emission peak, hence their relative emission between 300 and 400 nm is very small (\( f_{IR_{UV, TL-D}} = 0.03 \)). The spectral irradiance of fluorescent tubes is maximal in the long wavelength part.

The irradiance spectra were multiplied with the spectral sensitivities of BSF photoreceptors. Two photoreceptor classes were treated separately, R1-6 and R7&8, and the relative photoreceptor excitation was calculated for each class (Fig. 3C). Skylight and the LEDs strongly excite all short-wavelength photoreceptors (the blue and UV-blue sensitive R1-6, R7p and R7y), and to a lesser extent the green-sensitive R1-6. The opposite holds for the tubes that excite all short-wavelength receptors much less than skylight.

### 3.3. Influence of illumination on reproduction

Temperature (26.0 ± 0.3 °C) and relative humidity (70 ± 7%) was similar over treatments. The high intensity control was illuminated

<table>
<thead>
<tr>
<th>Illumination</th>
<th>Clutches (#, n = 6)</th>
<th>Larvae (#, n = 6)</th>
<th>Oviposition (day)</th>
<th>Longevity (days, n = 120)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LED-Low</td>
<td>5.8 ± 3.97</td>
<td>673 ± 498.6</td>
<td>16 ± 5.3</td>
<td>16 ± 0.7</td>
</tr>
<tr>
<td>LED-High</td>
<td>5.0 ± 4.34</td>
<td>766 ± 1142.7</td>
<td>13 ± 4.7</td>
<td>18 ± 0.7</td>
</tr>
<tr>
<td>Control-Low</td>
<td>4.7 ± 4.84</td>
<td>65 ± 75.0</td>
<td>11 ± 4.9</td>
<td>21 ± 0.7</td>
</tr>
<tr>
<td>Control-High</td>
<td>6.7 ± 5.72</td>
<td>257 ± 249.9</td>
<td>10 ± 3.5</td>
<td>17 ± 0.8</td>
</tr>
</tbody>
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Fig. 4. Number of larvae (mean ± SD) resulting from black soldier fly adults exposed to either LED or fluorescent tubes (Control).

Fig. 5. Survival curves of black soldier fly adults exposed to either LED light or fluorescent tube light (Control) at either low or high intensity.
with \( \approx 23 \text{ W} \text{ m}^{-2} \), whereas this was \( \approx 5 \text{ W} \text{ m}^{-2} \) in the low intensity control. The high intensity LED chamber was illuminated with \( \approx 24 \text{ W} \text{ m}^{-2} \) whereas this was \( \approx 14 \text{ W} \text{ m}^{-2} \) in the low intensity LED chamber. The number of clutches was similar over treatments (\( P = 0.903; \text{ Table 1} \)), but more egg clutches were found in the first round (\( P = 0.004 \)) than in the third round, whereas this was intermediate in the second round. Females in the low intensity control group oviposited earlier than females in the high intensity LED treatment (10 vs 16 days; \( P = 0.019 \)), whereas the two other groups were intermediate (\text{ Table 1}). Total number of larvae was affected by light treatment (\( P = 0.025 \)), but not by round (\( P = 0.379 \)). Scheffé post hoc testing did not detect differences between the four treatments (\( P = 0.331 \)). However, when comparing the two LED treatments with the two control treatments, the LED treatments resulted in more larvae (720 vs. 161; \( P = 0.006, \text{ Fig. 4} \)). Male and female longevity were similar (Mantel–Cox \( \chi^2 = 0.125, \text{ d.f.} = 1, P = 0.724 \)). However, animals in the low intensity control group lived longer than in the three other treatments (Mantel–Cox \( \chi^2 \geq 11.99, \text{ d.f.} = 3, P < 0.001, \text{ Fig. 5} \)).

4. Discussion

The major part of the BSF retina has high sensitivity in the short-wavelength part of the spectrum. An efficient illumination system should therefore produce substantial light output in the UV and blue. The visual system of the BSF is similar to that of other higher Diptera, except that their R1-6 photoreceptors occur in three regionalized versions with different spectral sensitivities. Because these cells can contribute to colour vision (Schnaitmann et al., 2013), the diverse R1-6 could support excellent trichromatic vision with UV, blue and green channels. The cells R7 and R8 provide BSF with at least dichromatic vision, based on a UV and a green channel.

Photoreceptor regionalization exists in different insect orders, and normally occurs with a high concentration of UV receptors in the dorsal retina (Gogala and Michieli, 1965; Horridge and Mclean, 1978; Stavenga, 1992, 2002; Stavenga et al., 2001). In the BSF, however, the situation seems to be reversed upside down; the dorsal retina contains predominantly green-sensitive R1-6, whereas UV-blue-sensitive R1-6 photoreceptors are concentrated in the ventral retina. If the receptor regionalization supports optimal contrasting, a BSF flying in front of foliage could be detected by a conspecific below as a black spot on a bright surface with the green-sensitive receptors dorsally, while the reflecting abdominal windows of a BSF walking on soil would appear to the UV-sensitive ventral receptors of a conspecific flying above as bright spots on a UV and blue-depleted background. Alternatively, a BSF resting on the lower side of a leaf in an upside-down position would observe the flying conspecifics on the sky-lit background with the UV-blue sensitive ventral part of the retina. The functional significance of such regionalization has yet to be elucidated by a careful analysis of the BSF natural visual environment. Another important implication is that the spatio-spectral characteristics of indoor breeding containers could be further optimized to better support the lekking behaviour.

The visually mediated behaviour of BSF could be obstructed by the flickering of artificial light sources. This problem was avoided by powering the LEDs and tube lighting with DC and high frequency-switching power supplies, respectively. The wavelengths of the LEDs were chosen to correspond to peak sensitivities of the different photoreceptors. Whereas the number of egg clutches was similar over treatments, more larvae were obtained when the adult BSFs were exposed to the LED lights than to the tube lights. It was not possible to count the number of eggs during the experiment without damaging them. However, the higher number of obtained larvae indicates a higher larval hatchability in the LED treatments, which might well have been caused by a higher frequency of mating, and hence a higher rate of fertilisation. Similarly, Tomberlin and Sheppard (2002) reported a lack of mating and obtaining only unfertilised eggs while using their artificial light sources. When their adult BSF were provided with direct sunlight, mating was observed and fertilized eggs were obtained. Therefore, in the context of BSF rearing, the spectral output of fluorescent tubes seemed inefficient and probably inadequately small in the UV part of the spectrum.

A single clutch contains approximately 320–620 eggs according to Pastor et al. (2015), although a range of 546–1505, averaging 998 eggs, has also been reported (Booth and Sheppard, 1984). Considering clutch size and the fact that each cage contained 10 females the total number of larvae obtained in all treatments was low.

The effect of light intensity on the number of larvae is difficult to interpret due to the large variation in the number of larvae. It has been suggested that no mating occurs below a light intensity of 63 \( \mu \text{mol m}^{-2} \text{s} \) and that most matings (75%) occur at light intensity above 200 \( \mu \text{mol m}^{-2} \text{s} \) (Tomberlin and Sheppard, 2002). However, it has also been suggested that mating activity decreases above 110 \( \mu \text{mol m}^{-2} \text{s} \) (Zhang et al., 2010). In both these studies BSF adults were exposed to sunlight, and these were conducted at similar latitudes (Texas, USA and Wuhan, China). Our results suggest that light spectral composition plays a key role in the production of fertile eggs, whereas the light intensity is of secondary importance.

The BSFs exposed to LED light produced more fertile eggs, which were oviposited later than those under fluorescent tube illumination. This could indicate that unfertilized eggs are oviposited earlier than fertilized eggs. Zhang et al. (2010) report a later peak in oviposition in BSFs exposed to sunlight, compared to BSFs exposed to a quartz-iodine lamp (17 vs. 13 days). Tomberlin and Sheppard (2002) reported a peak in oviposition four days after eclosion (73%) and did not observe oviposition later than six days after eclosion. Furthermore, they reported an adult longevity of 10–14 days when provided with water only, as did Zhou et al. (2013) for adults from three distinct BSF strains. In the latter study, strain differences were apparent regarding larval and pupal development as well as longevity. Our adults lived longer than in these studies, which could be due to strain differences. More likely it is explained by the provision of sugar water, serving as an energy source and thereby extending longevity. In the low intensity control BSFs lived longer than in the other treatments, which could be due to a lower activity level in this group, and hence a slower depletion of energy reserves.

This study shows that providing artificial light with wavelengths to which BSF adults' eyes are sensitive is an effective way to obtain fertilized eggs. Due to the relatively low efficiency of green and UV LEDs (or high efficiency of blue LEDs), our illumination system contained less long wavelength light than the standard CIE D65 (=sky-light) illuminant. In order to better approximate the natural illumination conditions, further studies could for instance use three times as much green LEDs (i.e. LED ratio UV:B:G = 1:1:3), thereby optimizing the visual pigment photoequilibrium and enhance the sensitivity of BSF photoreceptors. To further optimize BSF reproduction under indoor conditions, future studies should determine at which intensities the artificial lighting maximally induces mating and determine the optimal spatio-spectral distribution of light.

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