Title: *C. elegans* detect the color of pigmented food sources to guide foraging decisions

Authors: D. Dipon Ghosh[1], Xin Jin[1], and Michael N. Nitabach[1,2,3]*

Affiliations:

[1]Department of Cellular and Molecular Physiology, Yale University, New Haven, CT

[2]Department of Genetics, Yale University, New Haven, CT

[3]Program in Cellular Neuroscience, Neurodegeneration and Repair, Yale University, New Haven, CT

*Correspondence to: michael.nitabach@yale.edu

Abstract:

Here we establish that contrary to expectations, *Caenorhabditis elegans* nematode worms possess a color discrimination system despite lacking any opsin or other photoreceptor genes. We found that simulated daylight guides *C. elegans* foraging decisions with respect to harmful bacteria that secrete a blue pigment toxin. By absorbing yellow-orange light, this blue pigment toxin alters the color of light sensed by the worm, and thereby triggers an increase in avoidance of harmful bacteria. These studies thus establish the existence of a color detection system that is distinct from those of other animals. In addition, these studies reveal an unexpected contribution of microbial color display to visual ecology.

One-sentence summary:

Color detection contributes to *Caenorhabditis elegans* behavioral ecology by guiding foraging decisions.

Main text:

C. elegans live in decomposing organic matter where they feed on microorganisms (*1-3*), some of which secrete colorful pigments. While *C. elegans* lack any specialized photoreceptor cells or opsin genes, they possess an illuminance sensing system that mediates rapid escape responses to bright short-wavelength light (*4-6*). However, it is unknown whether *C. elegans* use light information, potentially including color, to inform complex decisions like foraging in environments containing colorful food sources. To address this question, we tested whether white light alters foraging decisions on *P. aeruginosa* bacterial lawns containing the blue pigment toxin pyocyanin, one of a number of small molecule phenazine toxins secreted by *P. aeruginosa* (*7-10*). Moderate intensity indirect daylight is typically 10-20 kilolux intensity and contains peaks in the blue and yellow-orange wavelength ranges (*11*). We selected an artificial LED array white light source with color temperature of 6500 K and 8 kilolux intensity to mimic these natural lighting conditions (**Fig. 1A,** see **fig. S1A**). Previous studies have shown that foraging decisions to remain on or leave a bacterial lawn are guided by a variety of factors (*12, 13*). Worms remain on bacterial lawns that are easy to eat and support its growth, while leaving lawns that are of poor nutritive quality, repulsive, or pathogenic (*12-17*). Worms are initially attracted to *P. aeruginosa* lawns, but over a time course of hours, as the *P. aeruginosa* continues to divide and secrete toxins, they respond to its increasingly pathogenic qualities and begin to leave (*15, 16, 18-21*). Whether light plays a role in guiding avoidance of *P. aeruginosa* has never been tested. Here we employed a standard lawn avoidance assay, placing worms on a *P. aeruginosa* lawn in the center of an agar plate, and quantified avoidance as the fraction of worms found off the lawn as a function of time (**Fig. 1A**). Consistent with prior studies, worms gradually avoid *P. aeruginosa* strain PA14 over a span of many hours (*15, 16, 18-21*) (**Fig. 1A**).

Surprisingly, however, this avoidance is dramatically potentiated by simulated daylight (**Fig. 1B**). This light has no effect on worms on lawns of non-toxic *E. coli* OP50 food bacteria, which they remain on without leaving (**Fig. 1B**).

Recent studies have implicated the *lite-1* gene – which encodes a non-canonical seven-transmembrane domain protein with closest homology to insect olfactory and gustatory chemoreceptors – in photophobic responses to bright short-wavelength light (*4-6*). To test whether the *lite-1* pathway is involved in light-dependent potentiation of PA14 avoidance, we compared avoidance of PA14 by *lite-1* null-mutant worms in the light and in the dark. Like wild-type worms, *lite-1* null-mutant worms gradually avoid PA14 over time, but their avoidance is unaffected by white light (**Fig. 1C**). These results indicate an essential role for the *lite-1* light response pathway in light-mediated potentiation of PA14 avoidance.

In order to determine whether the secreted blue toxin pyocyanin is involved in light-dependent potentiation of *P. aeruginosa* avoidance, we tested avoidance of a *phzM* mutant strain, PA14ΔphzM. This strain lacks the biosynthetic enzyme necessary for pyocyanin synthesis, but still synthesizes other phenazine toxins (*7-10, 22*), and PA14ΔphzM cultures are no longer blue (**Fig. 1D**). Simulated daylight has only a minimal effect on avoidance of PA14ΔphzM by wild-type worms, and no effect on avoidance by *lite-1* null-mutant worms (**Fig. 1, E and F**). The *phzM* mutation has pleiotropic effects beyond absence of pyocyanin, which include, among other things, increased production and secretion of other phenazines (*7*). The results thus far establish light-dependent potentiation of PA14 avoidance requiring both the *lite-1* light response pathway of the worm and the blue toxin pyocyanin secreted by *P. aeruginosa*.

To circumvent complexities associated with *P. aeruginosa* lawns, which continue to divide and secrete phenazines over the course of the assay, we tested whether pyocyanin is

sufficient to mediate light-dependent potentiation of avoidance of otherwise non-toxic bacteria. Accordingly, we employed lawns of non-toxic *E. coli* OP50 doped with pyocyanin (**Fig. 2A**). Wild-type worms avoid OP50 doped with 2.5 mM pyocyanin within one hour of placing on the lawn, but only in the presence of simulated daylight (**Fig. 2B**). This is more rapid than the avoidance of PA14 (see **Fig. 1B**), and is already at the maximum avoidance reached during extended exposure (**Fig. 2B**). This rapid, unchanging avoidance of OP50 lawns doped with 2.5 mM pyocyanin is likely due to the constant amount of pyocyanin present in the lawn, in contrast to *P. aeruginosa* lawns that continue to secrete pyocyanin over the course of the assay. In light of this unchanging avoidance of OP50 lawns doped with pyocyanin, for subsequent experiments we relied on this one hour time point. Like avoidance of PA14, light-dependent avoidance of OP50 + 2.5 mM pyocyanin is abolished by the *lite-1* null mutation (**Fig. 2C**). While doping with 0.25 mM pyocyanin fails to elicit avoidance, worms avoid OP50 + 5 mM pyocyanin both in the light and dark, and independently of *lite-1* function (**Fig. 2C**). These results indicate that an intermediate concentration of pyocyanin confers light-dependent and *lite-1*-dependent rapid avoidance of otherwise innocuous food bacteria, with higher concentrations conferring avoidance independent of light or *lite-1*.

lite-1 is expressed in a number of neurons in the worm nervous system, including the primary sensory neurons ASJ, ASI, and ASK (*5, 6, 23*). ASJ and ASI have been previously implicated in long-term avoidance of PA14 mediated by their direct responses to phenazines with Ca^{2+} increases and gene activation (*16*). In addition, *lite-1* expression in ASJ and ASK is sufficient for escape responses to short-wavelength light (*5, 6*). To determine the cellular locus of *lite-1* expression responsible for rapid light-dependent avoidance of OP50 + 2.5 mM pyocyanin, we employed *lite-1* null-mutant worms with targeted re-expression of *lite-1* in neurons ASJ,

ASH/ASI/PVQ, or ASK. Restoration of *lite-1* expression in ASJ or ASH/ASI/PVQ are each sufficient to rescue rapid light-dependent avoidance of OP50 + 2.5 mM pyocyanin, while restoration in ASK is not (**Fig. 2D**). These results localize *lite-1* function in rapid light- and pyocyanin-dependent bacteria avoidance to a small number of primary sensory neurons also involved in long-term avoidance of PA14, and which only partially overlap with those responsible for escape responses to short-wavelength light (**Fig. 2E**).

To test whether the blue color of pyocyanin independent of its toxic chemistry is sufficient to induce rapid avoidance of non-toxic OP50 lawns, we employed OP50 lawns doped with non-toxic inert blue food dye (**Fig. 3A**). Neither wild-type nor *lite-1* null-mutant worms avoid OP50 lawns doped with blue food dye, whether in dark or light, indicating that it is not solely the blue color of pyocyanin that drives light-potentiated rapid avoidance but also its chemical reactivity (**Fig. 3B**). One of the chemical features of pyocyanin that confers toxicity is that it enters eukaryotic cells and generates reactive oxygen species (ROS) through various mechanisms (*7-10, 22*). To determine if pyocyanin's combination of blue color and ROS-generating toxic chemistry underlies light-potentiated avoidance, we employed OP50 lawns doped with the colorless ROS-generating toxin paraquat (*24*) and non-toxic inert blue food dye (**Fig. 3C**). Wild-type worms rapidly avoid OP50 doped with 30 mM paraquat and blue dye, but only in the presence of light (**Fig. 3D**). This light-potentiated avoidance is abolished in *lite-1* null-mutant worms (**Fig. 3D**). As with OP50 + pyocyanin, higher concentrations of paraquat mediate rapid avoidance independent of both incident light and *lite-1*, while lower concentrations of paraquat with blue dye are insufficient to mediate avoidance (**Fig. 3D**). Doping OP50 lawns with 30 mM paraquat without any dye or with inert non-toxic red dye (**Fig. 3D**) are each insufficient to confer light-potentiated avoidance (**Fig. 3F**). These results indicate that rapid

light- and *lite-1*-dependent avoidance of pyocyanin-containing bacterial lawns relies both on its chemical reactivity as a generator of ROS and on its blue color. They also indicate that avoidance conferred by higher concentrations of pyocyanin relies solely on its ROS-generating capacity and is independent of *lite-1* function. This integration of color and chemical information to guide avoidance of food sources could enable more accurate discrimination of toxic from non-toxic lawns.

We hypothesized that blue pigment confers rapid light-potentiated avoidance of bacterial lawns containing ROS-generating toxins by absorbing long-wavelength light and thereby altering the spectral composition of light sensed by the worm. To test this possibility, we employed a series of shortpass and longpass optical filters to alter the spectra of incident light (corresponding photographs and spectra of filtered light are shown in **fig. S2**). Consistent with the previously determined action spectrum for *lite-1*-dependent photophobic responses (*4-6*), longpass filtered light lacking blue light fails to potentiate avoidance of OP50 + 2.5 mM pyocyanin (**Fig. 4A**). Surprisingly, however, light shortpass filtered with cut-offs of <500 nm or <550 nm also failed to potentiate avoidance (**Fig. 4A**). Only light shortpass filtered with <600 nm cut-off, which still includes the yellow-orange peak of 6500 K simulated daylight, was sufficient to potentiate avoidance of OP50 + 2.5 mM pyocyanin (**Fig. 4A**). This establishes that light-potentiated avoidance of bacterial lawns containing ROS-generating toxins and blue pigment requires not only short-wavelength blue light, but also long-wavelength yellow-orange light. This suggests the existence of a yellow-orange sensing pathway in addition to the *lite-1*-dependent blue light pathway.

Completely eliminating yellow-orange light abolishes light-dependent potentiation of avoidance of OP50 + 2.5 mM pyocyanin (**Fig. 4A**). Thus, we hypothesized that the presence of

blue pigment in the lawn reduces, but without eliminating, the yellow-orange content of light sensed by the worm, and that it is this decreased ratio of yellow-orange to blue light that potentiates avoidance of bacterial lawns containing ROS-generating toxins. It is uncertain exactly how blue pigment in its microenvironment alters the spectrum of light sensed by the worm. To circumvent this uncertainty, we eliminated blue pigment from the lawn and directly modified the spectral composition of light sensed by the worm with a "blue vinyl" filter that reduces without eliminating yellow-orange content to mimic the hypothesized effect of blue pigment (**Fig. 4B, fig. S2, A and B**). Remarkably, simulated daylight filtered through this blue vinyl filter recapitulates white light-potentiated rapid avoidance of OP50 + 30 mM paraquat, but now in the absence of blue pigment in the lawn (**Fig. 4C**). These results indicate that blue pigments confer light-potentiated avoidance of toxic bacterial lawns by absorbing yellow-orange light and thereby altering the spectral composition of light sensed by the worm. They also imply the existence of a yellow-orange light-sensing pathway that is required to be activated to potentiate avoidance of toxic lawns, but with activation of this pathway beyond some threshold preventing such potentiation.

As a further test of the hypothesis that it is the altered color of light, specifically the blue-to-yellow-orange ratio, that potentiates avoidance and not altered illuminance, we altered the intensity of simulated daylight (**fig. S3A**). Increasing white light intensity by ~3-fold to 25 klx failed to increase avoidance of OP50 + 30 mM paraquat (**fig. S3B**), supporting the hypothesis that it is the blue-to-yellow-orange ratio, and not illuminance, driving light-potentiated avoidance. While further increases to 50 klx or 100 klx did mediate increased avoidance of OP50 + 30 mM paraquat, these very high light intensities closer to that of direct sunlight (100-120 kilolux) identically increase avoidance of OP50 lawns without paraquat, most likely by

triggering *lite*-1-dependent escape responses (**fig. S3B**). Since *lite-1*-dependent responses to very bright light may themselves involve ROS (*23*), we used H2DCFDA, a fluorescent ROS indicator, to determine if intensities of light used in our experiments induce detectable ROS production in worms. No ROS signal is detected in worms placed on OP50 lawns doped with 30 mM paraquat in the dark or exposed to 8 klx white light for one hour (**fig. S3, C and D**). In contrast, substantial ROS signal is detected in worms on OP50 lawns exposed to 100 klx white light for one hour, regardless of whether paraquat is present (**fig. S3, C and D**). These results further support the conclusion that avoidance of toxic bacterial lawns potentiated by moderate intensity light operates by a different mechanism than photophobic responses to brighter light. They also rule out the possibility that blue pigment potentiates avoidance of bacterial lawns containing ROS-generating toxins by scattering blue light and thereby increasing activation of the *lite-1* short-wavelength light-sensing pathway.

Taken together, our results thus establish that *C. elegans* worms are capable of discriminating color in their environment by comparing activation of blue- and yellow-orange-sensitive pathways to guide foraging decisions on colorful toxic food sources. This reveals an unexpected mechanism by which color detection contributes to *C. elegans* behavioral ecology by heightening vigilance to ROS and thereby guiding foraging decisions on toxic bacteria (**Fig. 4D**). To our knowledge this is the first demonstration of the effect of microbial color display on an animal's foraging decision. Another example of color display by potential food sources influencing foraging decisions can be found in butterflies. Some non-toxic butterfly species mimic the coloration pattern of toxic butterflies in the same ecological niche to ward off predation (*25*). While selective pressure may have driven the worm's association between blue color and toxic food sources, blue color itself is insufficient to drive avoidance (see **Fig. 3, A**

186 **and B**). This raises the possibility that other non-toxic bacterial species may have co-opted blue
187 coloration to ward off being eaten, forcing worms to be more vigilant in the presence of blue
188 pigments while simultaneously considering information beyond color alone to avoid being
189 tricked by mimics.
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253 **Acknowledgments:**

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Figure 1. Light-potentiated avoidance of *P. aeruginosa* requires *lite-1* and pyocyanin blue pigment toxin.

A. Schematic of bacterial lawn avoidance paradigm. Worms (black squiggles) are placed on a lawn of *P. aeruginosa* strain PA14. Photographs are of *P. aeruginosa* PA14 liquid culture and 8 kilolux 6500 K white light with corresponding spectrum as measured by a CCD spectrometer. The fraction of worms outside the lawn is counted once per hour in the absence (dark) or presence (light) of 8 kilolux 6500 K white light simulating daylight illuminating the entire plate. **B.** Timecourse of wild-type worm avoidance of lawns of PA14 or *E. coli* OP50 in the presence of light or in the dark. Over the course of the nine hour assay, worms increase avoidance of PA14 in the light (orange) and dark (black), but this avoidance is greatly potentiated by white light. Worms remain on OP50 lawns (red) throughout the nine hour assay in the presence of light. **C.** Avoidance of PA14 by *lite-1* null-mutant worms is unaffected by white light. **D.** Schematic of lawn avoidance paradigm involving *P. aeruginosa* strain PA14ΔphzM, which is incapable of synthesizing pyocyanin. Photographs are of *P. aeruginosa* PA14ΔphzM liquid culture (note the change in color when pyocyanin is absent) and 8 kilolux 6500 K white light used in these experiments. **E.** White light has a less pronounced effect on avoidance by wild-type worms of mutant *P. aeruginosa* strain incapable of synthesizing pyocyanin. **F.** Avoidance of PA14ΔphzM by *lite-1* null-mutant worms is unaffected by white light. (Data represent an average of at least three assays with thirty worms per assay. All statistical comparisons are by two-way ANOVA with time as a repeated measure and post-hoc Bonferroni tests. For corresponding p and F values, see Supplemental Table 1. Error bars denote s.e.m. * indicates $p < 0.05$, ** indicates $p \leq 0.01$, and *** indicates $p \leq 0.001$.)

Figure 2. Light potentiates avoidance of non-toxic *E. coli* lawns doped with pyocyanin.

A. Schematic of experimental design for testing avoidance of *E. coli* OP50 lawns doped with pyocyanin. Photographs are of *E. coli* OP50 liquid culture and doping solutions used in these experiments. **B.** Timecourse of avoidance by wild-type worms of *E. coli* OP50 lawns doped with 2.5 mM pyocyanin in the presence or absence of light reveals white light potentiation of stable, long-term, avoidance of OP50 lawns. For corresponding *p* and F values, see Supplemental Table 1. **C.** White light potentiates rapid one-hour avoidance of OP50 lawns doped with 2.5 mM pyocyanin by wild-type but not *lite-1* null-mutant worms. Avoidance of OP50 lawns doped with 0.25 mM or 5 mM pyocyanin is unaffected by light. **D.** Re-expression of *lite-1* in *lite-1* null-mutant worms using the *trx-1* or *sra-6* promoters for expression in neurons ASJ and ASH/ASI/PVQ, respectively, rescues light-potentiated avoidance of OP50 lawns doped with 2.5 mM pyocyanin. Re-expression of *lite-1* using the *srg-8* promoter for expression in sensory neuron ASK has no effect. **E.** *lite-1* function in rapid light- and pyocyanin-dependent lawn avoidance localizes to a small number of primary sensory neurons previously shown to be involved in long-term PA14 avoidance, and only partially overlaps with those involved in escape responses to bright short-wavelength light. (Data in this figure and all remaining figures represent an average of at least three assays with thirty worms per assay. All statistical analyses were performed by one-way ANOVA with post-hoc Tukey-Kramer for pairwise comparisons or Bonferroni tests for comparisons with control. Error bars denote s.e.m. * indicates $p < 0.05$, ** indicates $p \leq 0.01$, and *** indicates $p \leq 0.001$.)

Figure 3. Light potentiates avoidance of *E. coli* lawns doped with paraquat colorless toxin mixed with non-toxic blue dye.

A. Experimental design for testing avoidance of OP50 lawns doped with non-toxic blue food dye. Photographs are of *E. coli* OP50 liquid culture and doping solutions used in these experiments. **B.** Avoidance of OP50 lawns doped solely with blue dye is unaffected by white light. **C.** Experimental design for testing avoidance of OP50 lawns doped with toxic paraquat and non-toxic blue dye. **D.** White light potentiates avoidance of OP50 lawns doped with 30 mM paraquat and blue dye by wild-type but not *lite-1* null-mutant worms. Avoidance of OP50 lawns doped with 20 mM or 40 mM paraquat and blue dye is unaffected by light. **E.** Experimental design for testing avoidance of OP50 lawns doped with toxic paraquat with no dye or with non-toxic red food dye. **F.** Avoidance of OP50 doped with 30 mM paraquat alone or paired with red dye is unaffected by white light.

Figure 4. Color of light determines avoidance of toxic bacterial lawns.

A. The spectral composition of light was modified by various optical filters. Photographs are of *E. coli* OP50 liquid culture and doping solutions used in these experiments, as well as lights modified by the indicated filter shining on a white background. Light spectra were measured using a CCD spectrometer. Blue light or blue-green light obtained with 500 nm or 550 nm cut-off shortpass filters each fail to potentiate avoidance of OP50 lawns doped with 2.5 mM pyocyanin. White light without blue light obtained with a 495 nm longpass filter also fails to potentiate avoidance. Only light containing both blue and yellow-orange light, obtained with a 600 nm cut-off shortpass filter, potentiates avoidance of OP50 doped with 2.5 mM pyocyanin. **B.** Experimental design for testing whether blue pigment causes light-potentiated avoidance by

absorbing long-wavelength light and altering the spectral composition of light sensed by the worm, by eliminating blue pigment from the lawn and directly controlling the spectral composition of light with filters. **C.** Light modified by a blue vinyl filter that decreases intensity of yellow-orange light without eliminating it potentiates avoidance of colorless OP50 lawns doped with 30 mM paraquat, but not lawns lacking paraquat. **D.** Schematic depicting the multisensory decision whereby integration of color and chemical information guides the foraging decision to stay on or leave toxic or non-toxic bacterial lawns. Blue pigment absorbs long-wavelength light and thereby alters the spectral composition of light detected by the worm. We propose that worms increase their vigilance to ROS when they detect a decrease in yellow-orange light content induced by blue pigments in their environment.

Supplementary Materials:

Supplementary Figure 1. Spectral composition of 6500 K 8 kilolux LED array white light source.

A. Photograph and spectral profile of white LED backlight as measured using a CCD spectrometer.

Supplementary Figure 2. Spectral composition of white light modified by filters.

A. Photographs of 8 klx white light modified by various filters. **B.** Spectral profiles of white light modified by LP (longpass) or SP (shortpass) filters with cut-offs at the indicated wavelengths were measured using a CCD spectrometer.

Supplementary Figure 3. Bright white light fails to potentiate avoidance of toxic colorless bacterial lawns.

A. Experimental design for testing whether increasing flux of blue light by increasing intensity of incident white light potentiates avoidance of colorless toxic food lawns. **B.** Increasing white light intensity ~3-fold to 25 klx fails to potentiate avoidance of OP50 lawns doped with 30 mM paraquat containing no blue dye. While further increases in white light intensity to 50 or 100 klx potentiate avoidance of OP50 lawns, this is independent of the presence of paraquat. **C.** Representative fluorescent images of the ROS-dependent indicator H2DCFDA in worms exposed to various intensities of white light for one hour, with or without 30 mM paraquat. **D.** Quantification of fluorescence from experiments shown in panel **c**. For each condition, at least 7 worms from 3 independent experiments were analyzed. Statistical analysis was performed by one-way ANOVA with post-hoc Tukey-Kramer for pairwise comparisons.

Supplemental Table 1. F-values and *p* values from statistical analyses performed by two-way ANOVA with time as a repeated measure.

Comparison	Relevant panel	F-value (time	*p* value (time)	F-value (condition)	*p* value (condition)
Wild-type worms on PA14 lawn, light vs. dark	Figure 1b	44.18	< 0.0001	27.57	0.0033
lite-1 null-mutant worms on PA14 lawn, light vs. dark	Figure 1c	52.93	< 0.0001	0.7630	0.4223
Wild-type worms on PA14ΔphzM lawn, light vs. dark	Figure 1e	27.04	< 0.0001	10.06	0.0248
lite-1 null-mutant worms on PA14ΔphzM lawn, light vs. dark	Figure 1f	26.31	< 0.0001	0.1715	0.6960
Wild-type worms on OP50 + 2.5 mM, light vs. dark	Figure 2b	0.4251	0.8891	462.3	0.0022

Materials and Methods:

Strains

C. elegans strains were maintained on Nematode Growth Medium (NGM) agar plates with *E. coli* OP50 as a food source. All strains were derived from the Bristol N2 wild-type strain. The *lite-1* null-mutant strain was backcrossed to wild-type N2 at least 5 times. Strains used for each figure are detailed below:

Figure 1: N2, KG1180 *lite-1(ce314)*

Figure 2: N2, KG1180 *lite-1(ce314)*, TQ1101 *lite-1 (xu7)*, TQ1179 *lite-1(xu7)*; xuEx301 [*sra-6p::lite-1::sl2::yfp; sra-6p::mCherry2; unc-122p::DsRed*], TQ1188 *lite-1(xu7)*; xuEx7 [*trxp-1::lite-1; unc-122p::gfp; trx-1p::mCherry2*], TQ1203 *lite-1(xu7)*; xuEx [*srg-8p::lite-1::sl2::yfp; unc-122p::gfp; srg-8p::mCherry2*]

Figure 3: N2, KG1180 *lite-1(ce314)*

Figure 4: N2

Supplementary Figure 3: N2

Lawn avoidance assay

P. aeruginosa lawn avoidance assays were performed on slow killing assay (SKA) plates as described (*8-10, 16, 18, 20*). *P. aeruginosa* cultures were grown in LB liquid media at 37^{o}C for 16-22 hours. 7 µL spots were added to the center of a 3.5 cm plate filled with 5 mL of SKA agar. Plates were first incubated at 37^{o}C for 22-24 hours, and then at room temperature for 16-20 hours. Worms were maintained in regular room lighting or incubator lighting conditions.

Growing worms in the presence or absence of light had no effect on assay results (data not shown). Thirty 18-24 hours post-L4 staged adult worms were transferred to the lawn for each assay. The fraction of worms out of those thirty that were off the lawn after the specified time interval was recorded as fraction avoiding.

Solutions of specified concentrations of pyocyanin (Cayman Chemical) and paraquat (ULTRA Scientific) were added to *E. coli* OP50 lawns on plates prepared exactly as described above. Erioglaucine (Alfa Aesar) or sulfarhodamine (MP Biomedicals) were used for blue and red color, respectively. Before adding solution, a 1.5 mL Eppendorf tube cut open at both ends was placed on the plate such that the rim of the tube circumscribed the boundary of the lawn. Next, 300 µL of the indicated solution was added to the tube to direct gradual application of solution to the lawn. Plates were prepared accordingly for three hours to allow for complete drying of the solution before transferring worms to the lawn and beginning the assay.

Statistical analysis

Sample sizes were chosen to ensure that at least two sets of experiments for each condition were carried out. No samples or animals were excluded from statistical analysis. Animals of a particular genotype were randomly assigned to assay plates. The order of assay plates loaded with worms was also randomized for each experiment, ensuring that the order of conditions tested was randomized. The investigator was blinded to genotype and condition (e.g., PA14 strain, concentration of paraquat) throughout, but not to different lighting conditions (e.g., light vs. dark, filter vs. no filter).

At least three independent assays were performed for each condition. For time course experiments in Figures 1 and 2, two-way ANOVA with time as a repeated measure and post-hoc Bonferroni tests was used to analyze statistical differences.

For all other experiments, one-way ANOVA was used to analyze statistical differences, with post-hoc Tukey-Kramer tests performed for pairwise comparisons between all conditions or post-hoc Bonferroni tests performed for comparisons to control. * indicates $p < 0.05$, ** indicates $p \leq 0.01$, and *** indicates $p \leq 0.001$.

Illumination

For experiments shown in Figures 1, 2, and 3, assay plates were illuminated in a completely dark room by a white LED backlight (Edmund Optics, Stock Number #83-873). After taking the lid off the plates, the plates were inverted onto the backlight. For assays performed in the dark, plates were positioned similarly either with the light off or on aluminum foil to block the light.

For experiments shown in Figure 4 and Extended Data Figure 3, plates were illuminated by a white LED (Thorlabs MCWHL5 with SM2P50-A attached) clamped to a ring stand. LEDs were positioned 18 cm above the plate, illuminating a 5 cm diameter spot covering the entire 3.5 cm diameter assay plate. Assay plates were always centered within this spot to circumvent any effects of imperfect collimation of the incident light. Multiple plates were illuminated simultaneously, each by a single LED. Multiple LEDs were powered simultaneously by a 4-Channel LED Driver (Thorlabs DC4100). In this lighting configuration, plate lids were removed, but plates remained upright.

A calibrated optometer Gamma Scientific (UDT Instruments Model S471 with 268P illuminance sensor) was used to measure illuminance. Irradiance at specified wavelengths was measured using the same instrument, but connected to a 268R sensor head that was continuously calibrated from 350 nm to 1100 nm. Irradiance at 470 nm was measured after filtering the light source through 500 nm shortpass filters.

The following filters were used in the experiments shown in Figure 4: 495 nm longpass filter (Thorlabs FGL495S), 500 nm shortpass filter (Edmund Optics Stock Number 64663), 550 nm shortpass filter (Edmund Optics Stock Number 64664), 600 nm shortpass filter (Edmund Optics Stock Number 64665), and a blue vinyl sheet (Hull's Art store). Spectral profiles were measured using a compact CCD spectrometer (Thorlabs CCS100 with connected cosine corrector CCSB1). Relative intensities measured by the spectrometer do not permit comparisons of absolute intensity between the various filters. Illuminance of white light modified by each filter was calibrated to ensure that the intensity of short-wavelength (470 nm) light passing through each filter was identical as measured using a calibrated irradiance detector described above. This only applied to white light modified by the blue vinyl filter, which required calibration of < 1 klx.

ROS detection

After exposure to the specified light/dark and paraquat conditions, worms were washed three times with M9 buffer. Seven to nine worms from each treatment group were incubated with 25 µM 2'-7'-dichlorodihydrofluorescein (Biotium) in the dark for 30 min at 20 °C. After H2DCFDA incubation, worms were washed with M9 solution and mounted in PBS buffer containing 1 mM sodium azide. Fluorescence was measured immediately after mounting to slides using a Leica M165FC fluorescent stereomicroscope at excitation/emission wavelengths of

488 nm and 520 nm. The mean fluorescence intensity of the whole body of the worm was quantified using ImageJ software. Values were then compared using one-way ANOVA with post-hoc Tukey-Kramer test. For each condition, at least 7 worms from 3 independent experiments were analyzed.

Additional Author notes:

Author Contributions

Designed study and experiments: DDG, MNN

Performed experiments: DDG, XJ

Data analysis and statistics: DDG, XJ

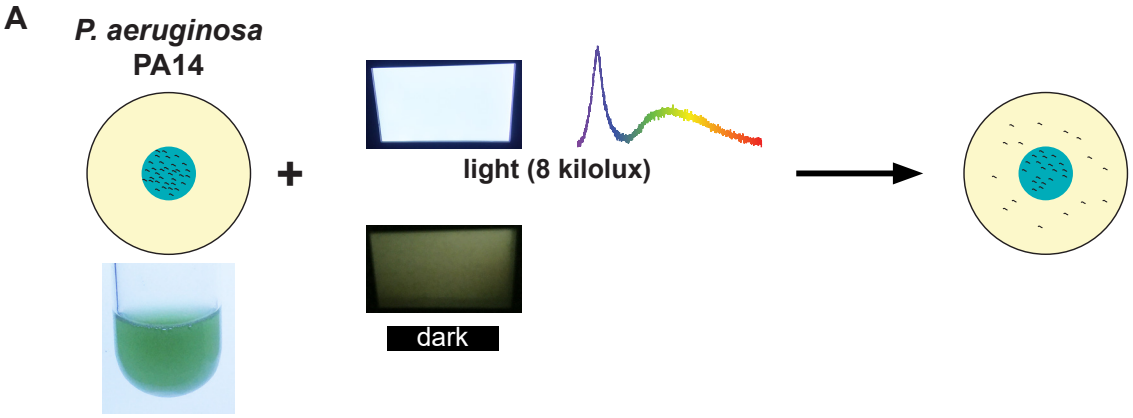
Wrote the paper: DDG, MNN

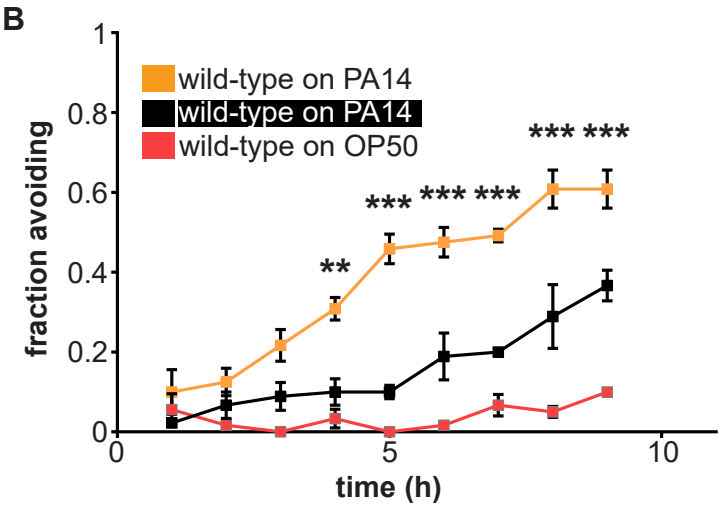
Author Information

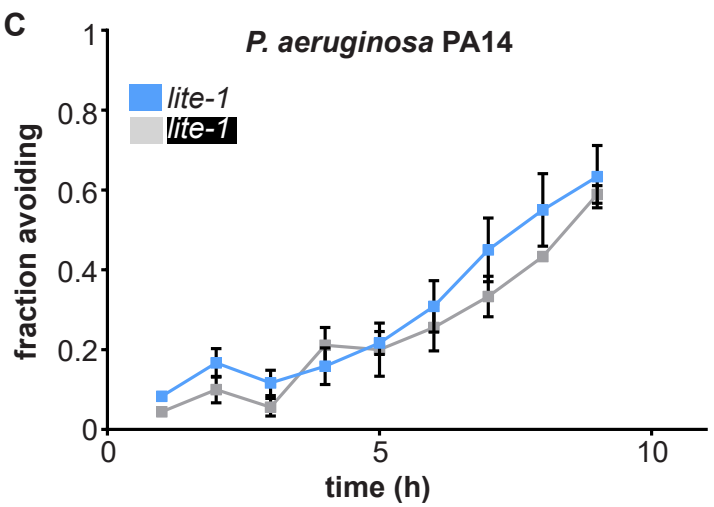
The authors declare no conflicts of interest.

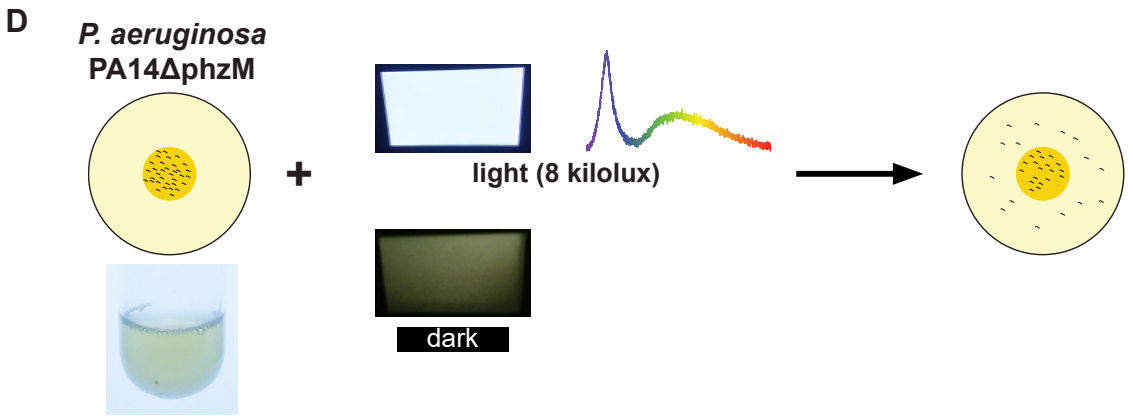
Correspondence and requests for material should be addressed to michael.nitabach@yale.edu

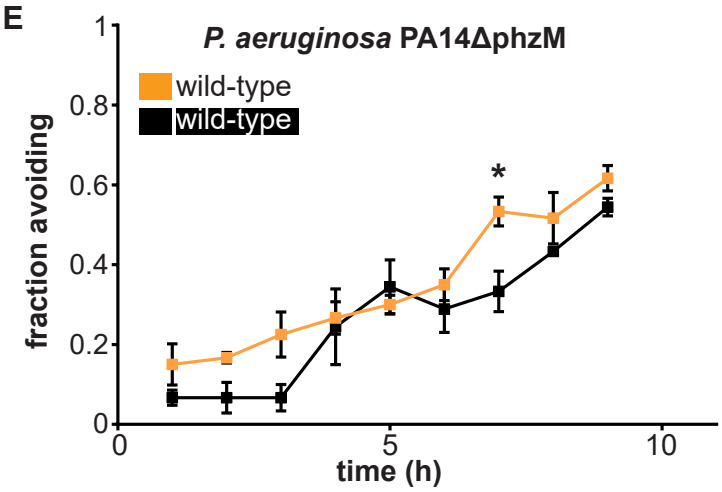
Figure 1











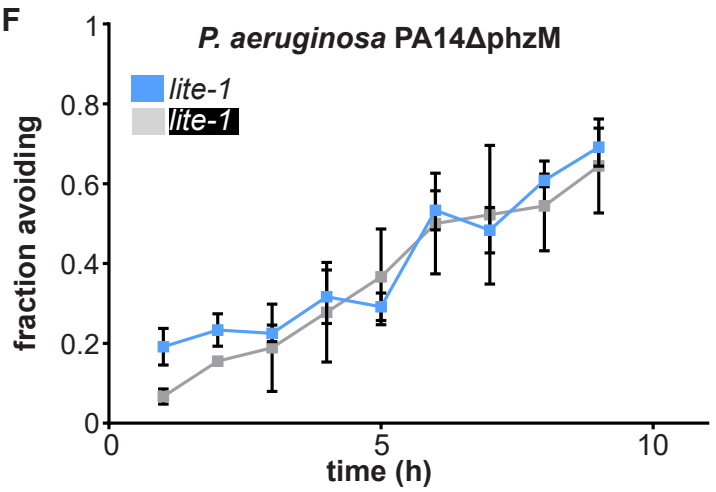


Figure 2

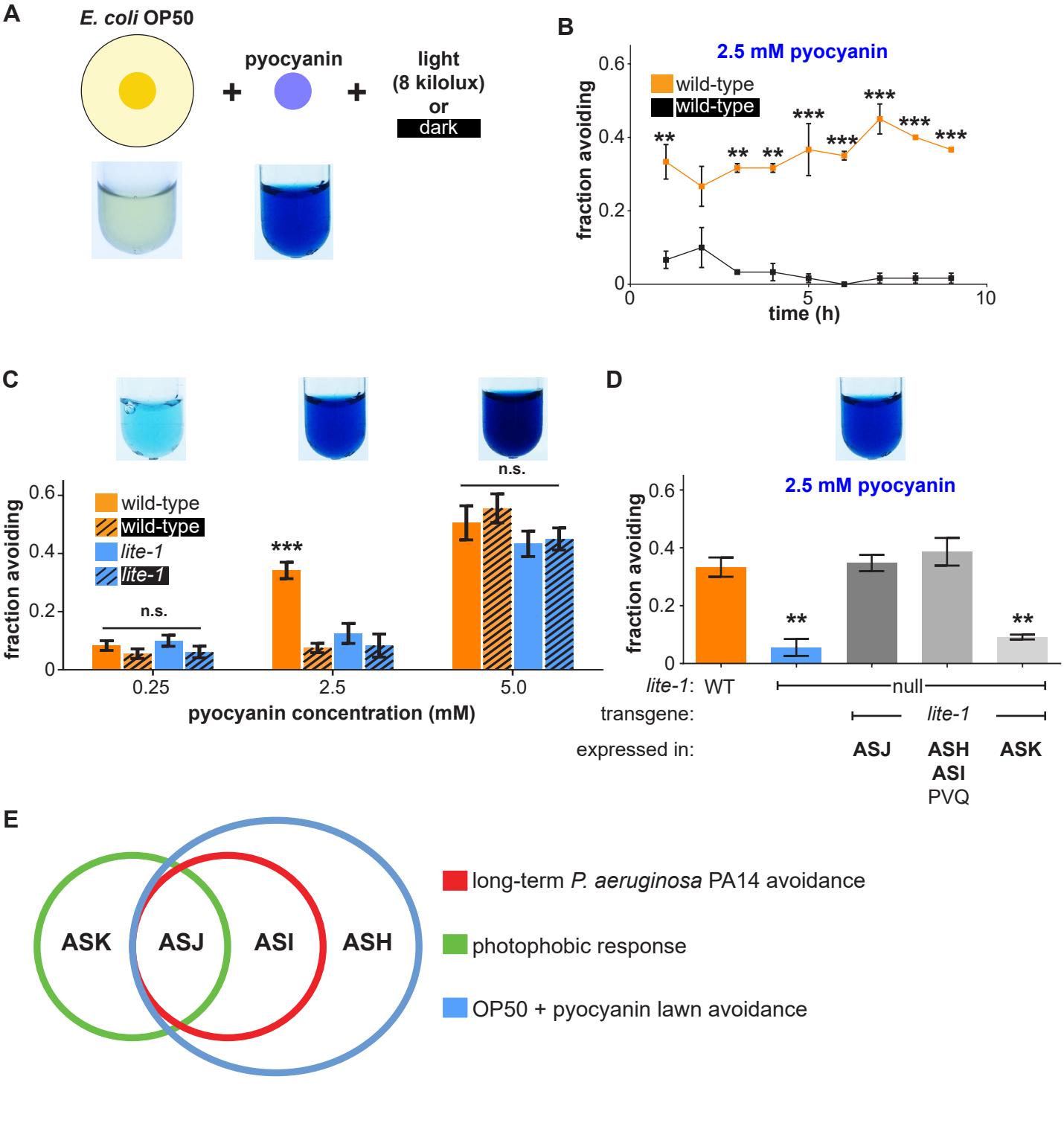


Figure 3

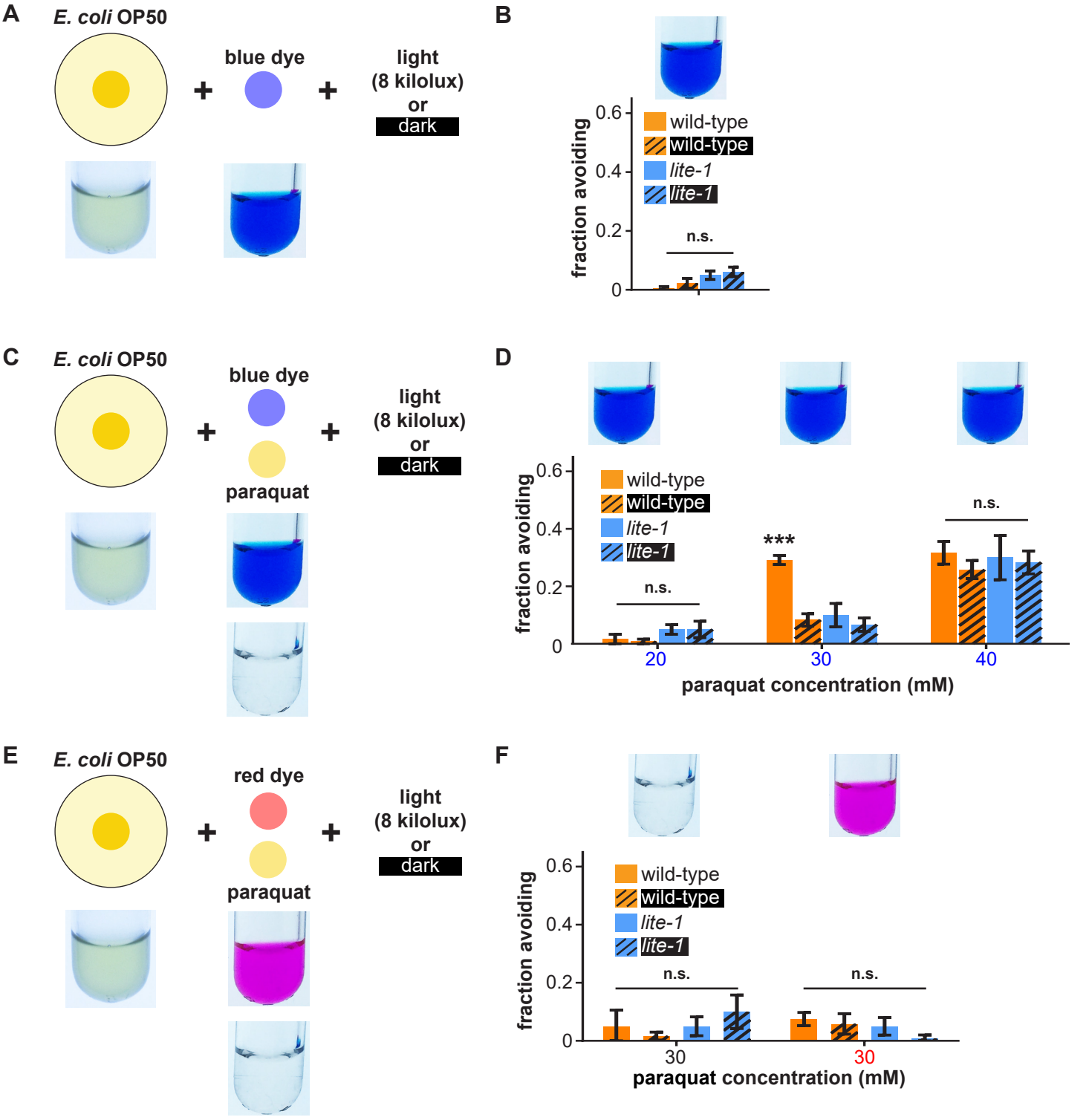
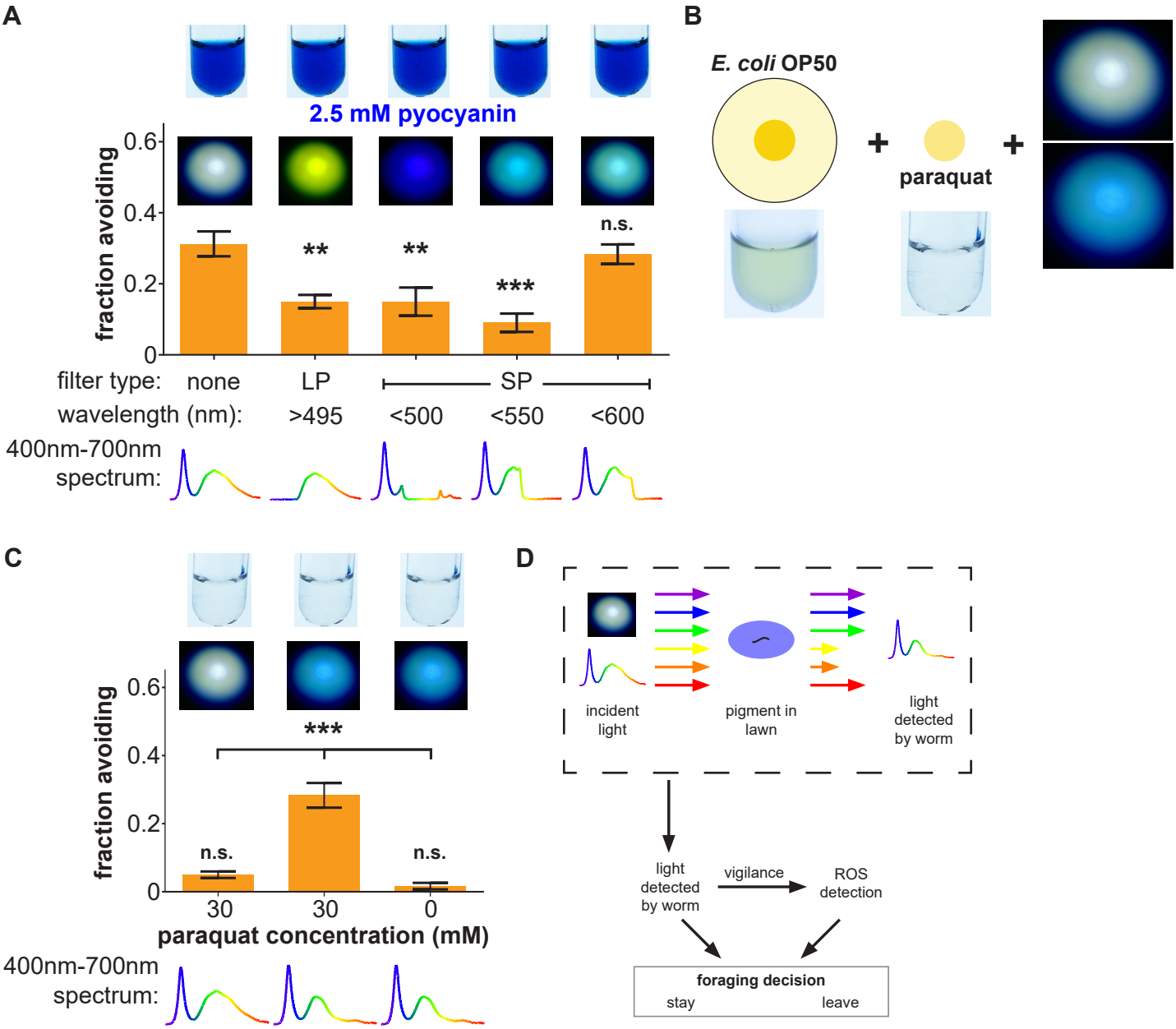


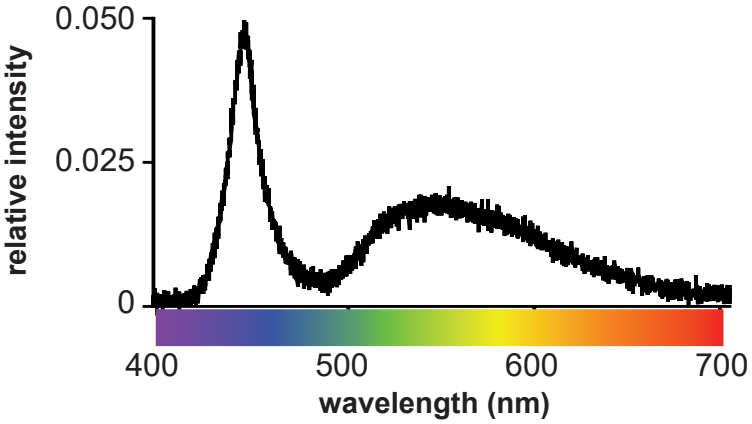
Figure 4



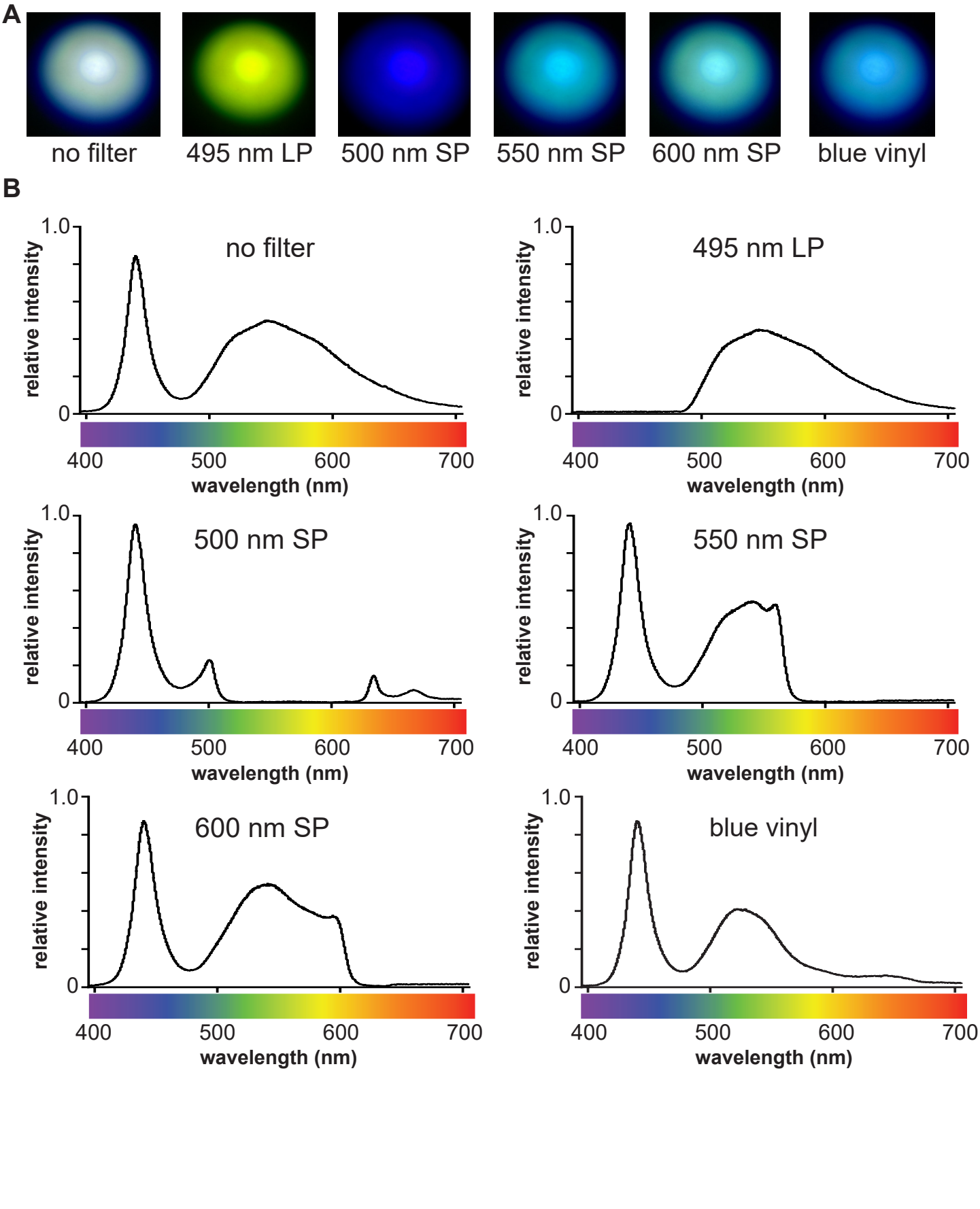
Supplementary Figure 1

A

8 kilolux LED array white light source (Color temp.: 6500 K)



Supplementary Figure 2



Supplementary Figure 3