

## SENSORY PERCEPTION

*C. elegans* discriminates colors to guide foragingD. Dipon Ghosh<sup>1,2\*</sup>, Dongyeop Lee<sup>2</sup>, Xin Jin<sup>1†</sup>, H. Robert Horvitz<sup>2</sup>, Michael N. Nitabach<sup>1,3,4\*</sup>

Color detection is used by animals of diverse phyla to navigate colorful natural environments and is thought to require evolutionarily conserved opsin photoreceptor genes. We report that *Caenorhabditis elegans* roundworms can discriminate between colors despite the fact that they lack eyes and opsins. Specifically, we found that white light guides *C. elegans* foraging decisions away from a blue-pigment toxin secreted by harmful bacteria. These foraging decisions are guided by specific blue-to-amber ratios of light. The color specificity of color-dependent foraging varies notably among wild *C. elegans* strains, which indicates that color discrimination is ecologically important. We identified two evolutionarily conserved cellular stress response genes required for opsin-independent, color-dependent foraging by *C. elegans*, and we speculate that cellular stress response pathways can mediate spectral discrimination by photosensitive cells and organisms—even by those lacking opsins.

The roundworm *C. elegans* lives in decomposing organic matter such as compost heaps, where it feeds on microorganisms (1, 2), some of which secrete colorful pigments (3–5). *C. elegans* lacks the spe-

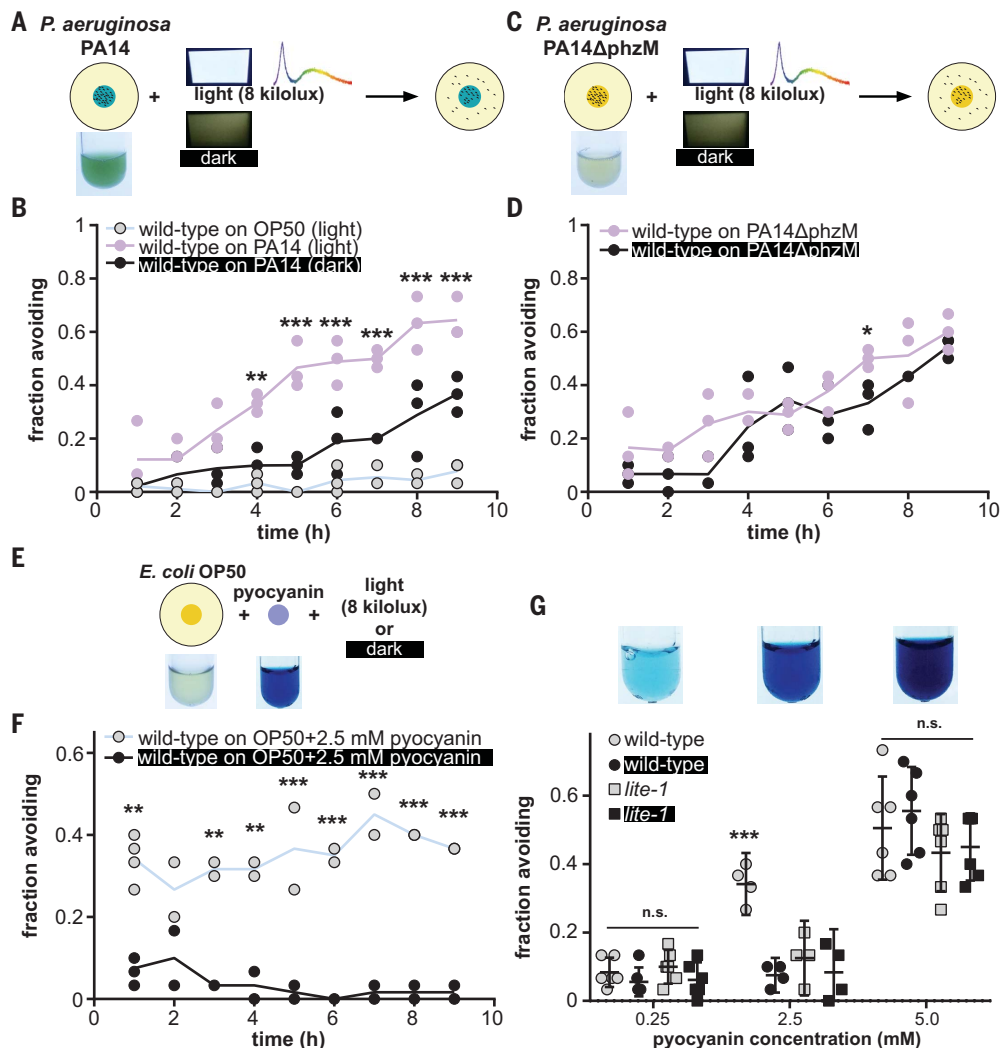
cialized eyes, photoreceptor cells, and opsin genes that underlie canonical visual-system functions (6–8). Nevertheless, *C. elegans* can detect and respond to short-wavelength light, including blue light, using the LITE-1 and

GUR-3 proteins, which are similar to insect gustatory chemoreceptors (9–14). Although it has been shown that visible light can influence *C. elegans* physiology (15) and behavior (9–14), whether microbial pigments affect *C. elegans* foraging has not been previously addressed.

We investigated whether white light alters *C. elegans* strain N2's avoidance of pathogenic *Pseudomonas aeruginosa* PA14 bacterial lawns secreting the blue pigment pyocyanin, a reactive oxygen species (ROS)-generating toxin (Fig. 1A and fig. S1A) (16–23). White light markedly potentiated the gradual avoidance of PA14 but not of nontoxic *Escherichia coli*

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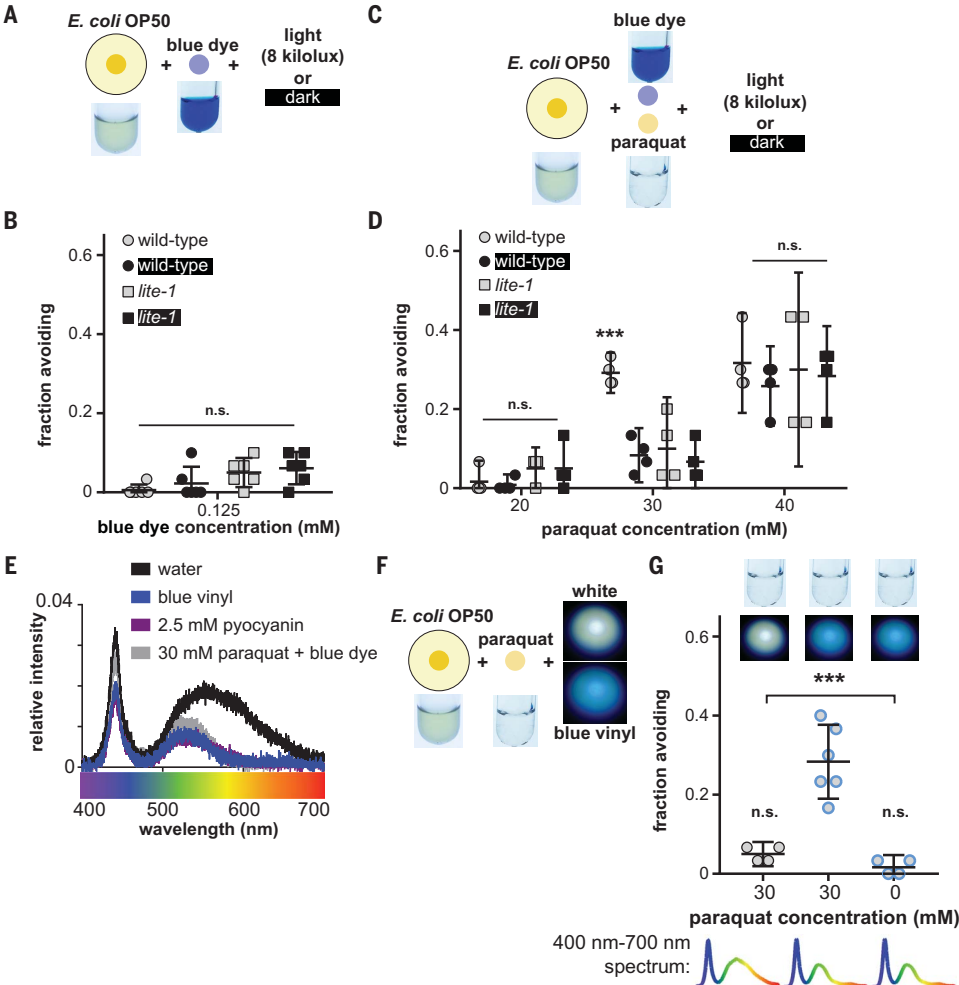
**Fig. 1. Blue-pigment toxin pyocyanin underlies light-potentiated avoidance of *P. aeruginosa*.** (A) Schematic depicting worms ( $n = 30$  per assay) on a lawn of *P. aeruginosa* strain PA14 in the absence (dark) or presence (light) of 8 kilolux of white light. All photographs shown in this and the following figures are of example liquid cultures or test solutions used in the experiment and of lights, modified as indicated, shining on a white background. (B) Time course of wild-type worm avoidance of lawns of PA14 in the light (purple) and dark (black) or of *E. coli* OP50 in the light (gray). Lines represent the average of assays individually depicted by data points.  $n = 3$  lawn avoidance assays. (C and D) Time course of wild-type worm avoidance of PA14 $\Delta$ phzM lawns in light (purple) and dark (black). PA14 $\Delta$ phzM is incapable of synthesizing pyocyanin (note, these cultures are not blue).  $n = 3$ . (E and F) Time course of avoidance by wild-type worms of *E. coli* OP50 lawns supplemented with 2.5 mM pyocyanin in the presence (gray) or absence (black) of light.  $n = 4$  for measurement at 1 hour;  $n = 2$  for measurements at 2 to 9 hours. (G) One-hour avoidance of OP50 lawns supplemented with 0.25, 2.5, or 5 mM pyocyanin by wild-type (circles) and *lite-1* null-mutant (squares) worms in either the presence (gray) or absence (black) of light. Data for wild-type avoidance of lawns supplemented with 2.5 mM pyocyanin in (F) and (G) were from the same experiments. All statistical comparisons for time course



experiments were by two-way analysis of variance (ANOVA) with time as a repeated measure and post-hoc Bonferroni tests. For *P* and *F* values, see table S1. Experiments are single time points (1 hour) unless otherwise indicated. Statistical analyses were performed by one-way ANOVA with post-hoc Tukey-Kramer tests for pairwise comparisons or Dunnett or Bonferroni tests, as appropriate, for comparisons with control. Error bars denote 95% confidence intervals. \* $P \leq 0.05$ ; \*\* $P \leq 0.01$ ; \*\*\* $P \leq 0.001$ ; n.s., not significant.

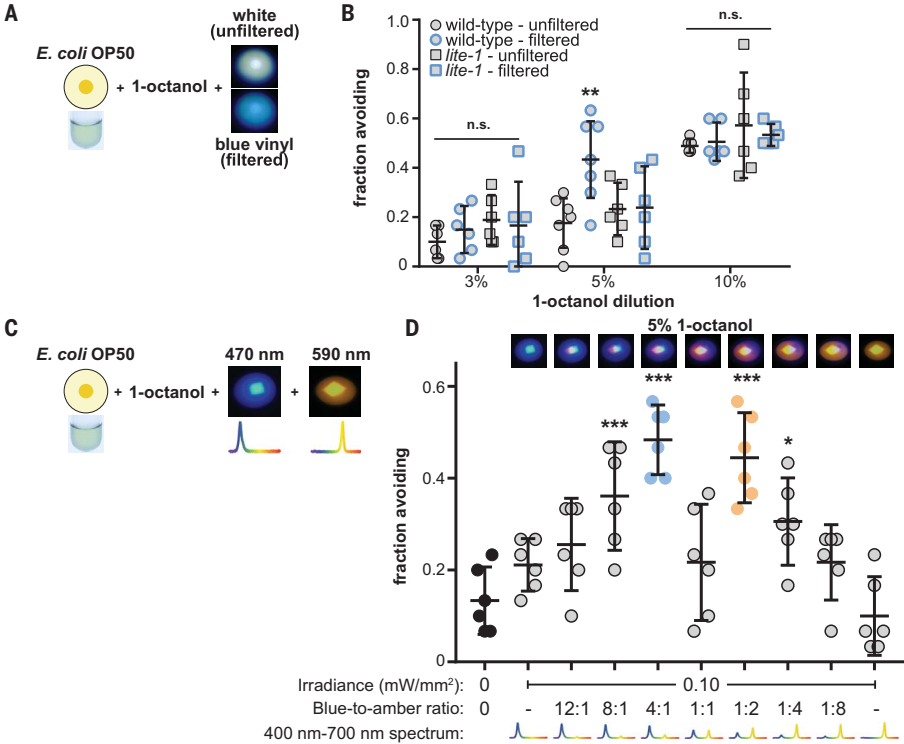
**Fig. 2. Spectral content potentiates avoidance of toxic bacterial lawns.**

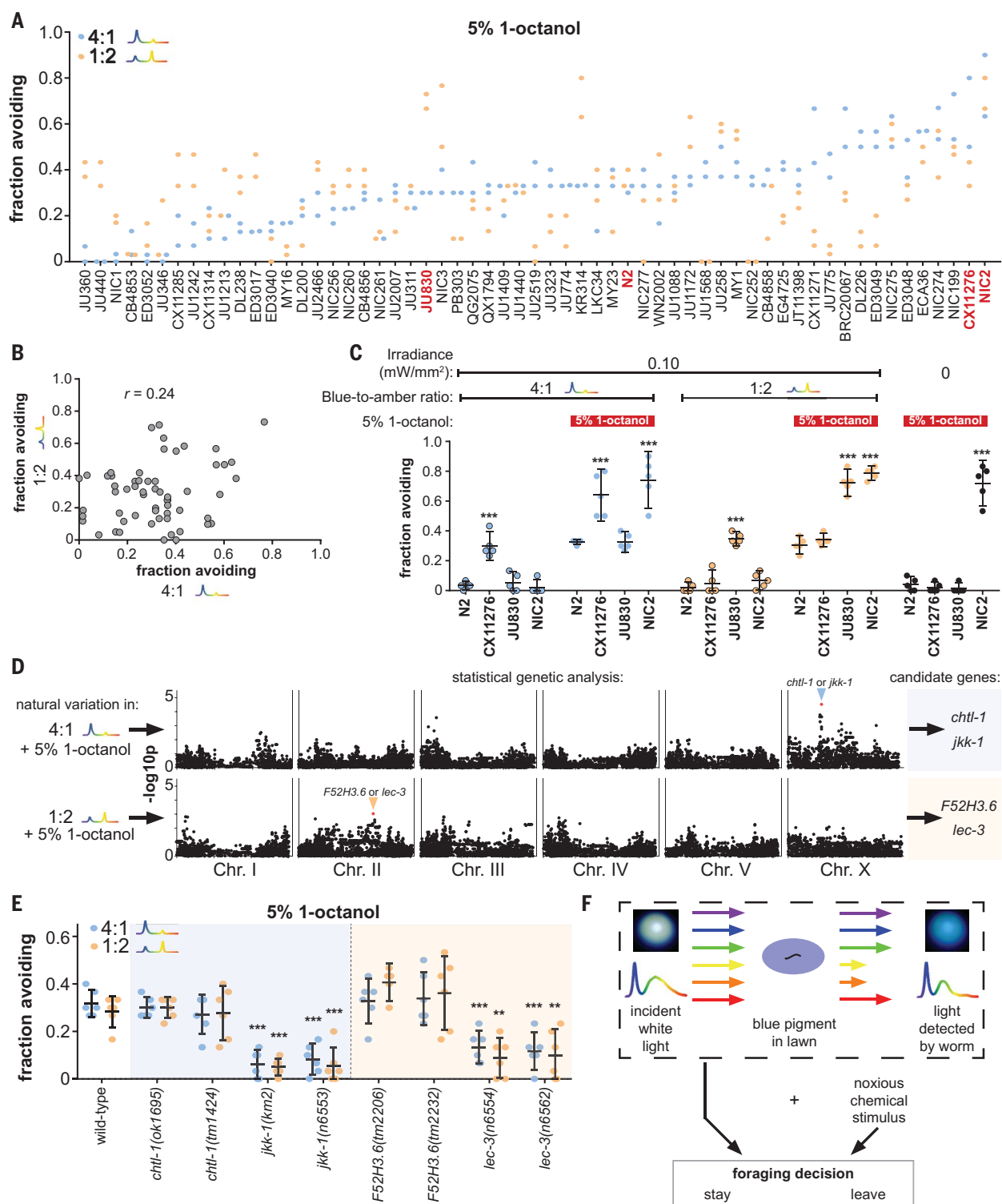
(A and B) Avoidance of OP50 lawns supplemented with 0.125 mM blue dye. (C and D) Avoidance of OP50 lawns supplemented with 0.125 mM blue dye and specified concentrations of paraquat (20, 30, and 40 mM). (E) Comparison of the spectra of white light filtered with water (black), blue vinyl filter (blue), 2.5 mM pyocyanin solution (purple), or 30 mM paraquat and blue dye solution (gray). (F and G) Avoidance of OP50 lawns supplemented with or without paraquat in the presence (gray with blue borders) or absence (gray) of a blue vinyl filter modifying the color of the white light.



**Fig. 3. Foraging is guided by the relative intensities of blue and amber light.**

(A and B) Avoidance of OP50 lawns in the presence of the specified dilutions of 1-octanol (3, 5, and 10%) and in the presence (gray with blue borders) or absence (gray) of the blue vinyl filter. (C and D) Avoidance of OP50 lawns in the presence of 5% 1-octanol and incident light of different colors composed of the specified ratios of narrow-band blue and amber light.





**Fig. 4. Evolutionarily conserved genes *jkk-1* and *lec-3* are required for naturally varying color-dependent foraging.** (A) Avoidance of OP50 lawns by 59 wild *C. elegans* strains in the presence of 5% 1-octanol and 4:1 or 1:2 blue-to-amber light. N2, CX11276, JU830, and NIC2 strains are indicated in red.  $n = 2$ . (B) Correlation of lawn avoidance in the presence of 5% 1-octanol and 4:1 and 1:2 blue-to-amber light by each wild strain. Pearson's correlation coefficient  $r(57) = 0.24$ ;  $P = 0.063$ . (C) Avoidance of OP50 lawns by N2, CX11276, JU830, and NIC2 strains with or without 5% 1-octanol and 4:1 or 1:2 blue-to-amber light, as specified. (D) Statistical genetics analysis with

highest-scoring polymorphisms (indicated by red dots) and neighboring high-confidence polymorphisms that suggested the candidate genes *cht1-1* and *jkk-1* [blue arrowhead and blue box in (E)] and *F52H3.6* and *lec-3* [orange arrowhead and orange box in (E)]. (E) Avoidance of OP50 lawns in the presence of 5% 1-octanol and 4:1 or 1:2 blue-to-amber light by *cht1-1*, *jkk-1*, *F52H3.6*, and *lec-3* null-mutant worms. (F) Blue pigment absorbs long-wavelength light and thereby alters the spectral composition of light detected. The integration of color and chemical information guides worms' foraging decision to stay on or leave bacterial lawns.

strain OP50 (Fig. 1B). *lite-1* null-mutant worms also avoided PA14, but their avoidance was unaffected by white light (fig. S1B). We also examined the worms' avoidance of a *P. aeruginosa* mutant strain, PA14ΔphzM, that cannot synthesize pyocyanin but still synthesizes other nonblue ROS-generating toxins (19). PA14ΔphzM cultures were not blue (Fig. 1C), and light only minimally affected the avoidance of PA14ΔphzM by wild-type or *lite-1* null-mutant worms (Fig. 1D and fig. S1C). These results demonstrate that light-dependent potentiation of PA14 avoidance requires both *lite-1* and pyocyanin.

By supplementing lawns of nontoxic OP50 with pyocyanin (Fig. 1E), we found that pyocyanin is sufficient to confer light- and *lite-1*-dependent avoidance on otherwise innocuous bacteria (Fig. 1, F and G). We tested whether a chemically inert blue dye, spectrally matched (fig. S2A) to pyocyanin, coupled with the colorless ROS-generating toxin paraquat (fig. S2B) would support light-potentiated avoidance. Light potentiated wild-type but not *lite-1* avoidance of OP50 supplemented with both blue dye and paraquat, but not with either independently (Fig. 2, A to D, and fig. S2, C to F). This observation indicates that avoidance of pyocyanin-containing lawns relies on both pyocyanin's chemical and spectral properties.

Using optical filters (fig. S3, A and B), we found that eliminating short-wavelength blue or long-wavelength amber light disrupted light-potentiated avoidance (fig. S3C). By contrast, directly filtering incident white light through a blue vinyl filter that increased the blue-to-amber ratio to match the spectral properties of pyocyanin potentiated avoidance of OP50 supplemented with paraquat without blue pigment (Fig. 2, E to G). These results indicate that blue pigments enhance avoidance by changing the spectrum of light in the worm's environment (fig. S4). Blue vinyl-filtered light also potentiated avoidance of OP50 lawns in the presence of the aversive odorant 1-octanol (24), which suggests that color might generally influence the avoidance of aversive stimuli (Fig. 3, A and B, and fig. S5A).

To analyze spectral influences on foraging, we tested combinations of monochromatic blue and amber light sources for potentiation of OP50-lawn avoidance in the presence of 1-octanol (Fig. 3C). Although neither pure blue nor pure amber light potentiated lawn avoidance, mixed colors differentially potentiated avoidance depending on the blue-to-amber ratio (Fig. 3D). This observation indicates that the relative intensities of blue and amber visible light guide foraging decisions and establishes that *C. elegans* can discriminate colors.

Next, we investigated whether *C. elegans* strains that are independently isolated from the wild, and presumably adapted to diverse ecological niches (25–27), exhibit variation in color-dependent foraging. We detected sub-

stantial variation in 4:1 and 1:2 blue-to-amber spectrum-dependent foraging among 59 wild strains (Fig. 4A). Notably, 4:1 and 1:2 blue-to-amber ratio sensitivities were uncorrelated (Fig. 4B), which suggests that complex, potentially distinct mechanisms underlie color-specific sensitivities. For example, compared with N2, strains CX11276 and JU830 exhibited relatively heightened sensitivities to 4:1 blue-to-amber light and 1:2 blue-to-amber light, respectively, whereas NIC2 maximally avoided the lawn regardless of color (Fig. 4A). To dissect how spectral discrimination and 1-octanol avoidance contribute to color-dependent foraging, we tested the avoidance of lawns illuminated with 4:1 and 1:2 blue-to-amber light, with and without octanol, by these strains. CX11276 and JU830 were sensitive to specific colors even without 1-octanol, whereas NIC2 avoided lawns with 1-octanol even without light (Fig. 4C). Thus, whereas color-dependent lawn avoidance by N2, which is relatively insensitive to colors, requires the presence of an additional aversive stimulus, for more color-sensitive strains like CX11276, color-specific illumination is sufficient. These results demonstrate that naturally varying color and odorant sensitivities drive strain differences in foraging.

Using approaches adapted from statistical genetics (27), we determined that no single genomic polymorphism could causally account for the observed variation in color-dependent foraging (Fig. 4D). However, by considering multiple neighboring single-nucleotide polymorphism (SNPs) within a given genomic region (28), we identified two sets of two genes—*cht1-1* and *jjk-1* as well as *F52H3.6* and *lec-3*—in the regions of the two highest-scoring polymorphisms that contribute to variation in the avoidance of lawns under 4:1 and 1:2 blue-to-amber light, respectively (Fig. 4E and fig. S6). Color-dependent avoidance was abolished in two independent null-mutant strains of *jjk-1* and in two independent null-mutant strains of *lec-3*, whereas color-dependent foraging by *cht1-1* and *F52H3.6* null-mutant worms was unaffected (Fig. 4E). Loss of *jjk-1* or *lec-3* function did not impair avoidance responses to brighter blue light or higher 1-octanol concentrations (fig. S7). These results revealed that *jjk-1* and *lec-3* contribute to color-dependent foraging.

We established that *C. elegans* discriminates colors, and we identified two genes required for color-dependent foraging. Mammalian homologs of *jjk-1* and *lec-3*—MKK7, an activator of c-Jun N-terminal kinases (JNKs) (29), and galectin, a member of a protein family that binds beta-galactoside sugars (30), respectively—can interact in mediating cellular responses to stressors, including ultraviolet light (31–37). Genes like *jjk-1* and *lec-3* might function in opsin-independent, spectrally sen-

sitive stress response pathways to guide *C. elegans* foraging decisions on food sources that vary in color and toxicity (Fig. 4F). The functions of microbial pigmentation are poorly understood (38). We suggest that pigmentation contributes to evolving interactions between pathways underlying the synthesis and secretion of pigmented factors by microbes and the responses to these pigments by foraging hosts like *C. elegans*.

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## ACKNOWLEDGMENTS

We thank S. Xu, E. Andersen, S. Mitani, and the Caenorhabditis Genetics Center for *C. elegans* strains; F. Ausubel and L. Dietrich for *P. aeruginosa* strains; and members of the Nitabach, Horvitz, and Kazmierczak laboratories for technical support, advice, and comments. **Funding:** D.D.G. is a Howard Hughes Medical Institute Fellow of the Life Sciences Research Foundation. Work in the laboratory of H.R.H. was supported by the National Institute of

General Medical Sciences (NIGMS), NIH (R01GM024663). H.R.H. is an investigator of the Howard Hughes Medical Institute. Work in the laboratory of M.N.N. is supported in part by NIGMS, NIH (R01GM098931). **Author contributions:** D.D.G. initiated the study. D.D.G., D.L., and X.J. performed experiments; D.D.G., D.L., X.J., H.R.H., and M.N.N. designed experiments and analyzed data. D.D.G, H.R.H, and M.N.N. wrote the paper with input from the other authors. **Competing interests:** The authors declare no

competing interests. **Data and materials availability:** All data are available in the manuscript or the supplementary materials.

SUPPLEMENTARY MATERIALS

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Materials and Methods  
Figs. S1 to S7

Table S1  
References (39, 40)  
MDAR Reproducibility Checklist

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12 June 2020; accepted 8 January 2021  
10.1126/science.abd3010

## ***C. elegans* discriminates colors to guide foraging**

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*Science* **371** (6533), 1059-1063.  
DOI: 10.1126/science.abd3010

### **A new way to "see" color**

Color perception is an important aspect of the way many organisms navigate their world. The ability to perceive color has thus far thought to depend on the presence of either eyes or minimally receptive cells containing opsin receptor genes. Ghosh *et al.* show that foraging *Caenorhabditis elegans* roundworms, which do not have eyes or opsins, can distinguish a blue color indicative of a toxin released by bacterial mats (see the Perspective by Neal and Vosshall). They suggest that the worms do this through the detection of the ratio between blue and amber light, a process dependent on at least two cellular stress-response genes. Different strains of *C. elegans* responded to different ratios, suggesting that this pathway plays an ecological role.

*Science*, this issue p. 1059; see also p. 995

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