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Negative phototaxis in *M. incognita*

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Abstract

Phototaxis is a well-known behavior in many animals. However, little is known whether nematodes living in soil recognize light. *Meloidogyne incognita* is one of the most important root-knot nematode pests in the world. It has a very wide host range and can infect almost all plant species. Here, we examined the phototactic response in *M. incognita*. Negative phototactic behavior was observed in *M. incognita* on an agar plate. Nematodes responded by avoiding white light and blue light more than red light on agar plates. Light avoidance behavior of the nematodes was examined by microscopic analysis, and the nematodes escaped from near ultraviolet light (365 nm) in only approximately 3.2 seconds. They also avoided blue (470 nm), blue-green (500 nm), and green (550 nm) light in approximately 4.1, 7.4, and 10.0 seconds, respectively. These results suggest that *M. incognita* exhibits negative phototaxis, in particular, responding efficiently to shorter wavelength of light.

Keywords: M. incognita, nematode, root knot, negative phototaxis

1. Introduction

Phototaxis is a common behavior in many animals. In many cases, phototaxis is important for animal survival. However, animals living in darkness sometimes lack the ability to respond to light. Approximately 50% of marine nematodes are thought to have photoreceptors (McLaren, 1973). *Oncholaimus vesicarius* and *Enoplus anisospiculus* are reported to have negative phototaxis to blue light (Burr., 1979). However, it is not well known whether *M. incognita* living in soil recognize light.

The nematode *C. elegans* is reported to possess light-sensitive neurons with CNG channel-mediated phototaxis (Cho et al., 2005; Ward et al., 2008). Furthermore, some marine and freshwater nematodes, *O. vesicarius* and *E. anisospiculus* contain pigments in their heads and are phototactic, though no molecular data are available because of limited genome information (Chitwood & Murphy, 1964; Croll, 1966; Burr, 1979).

One of the major nematode pests, *M. incognita*, causes great damage to agricultural production and lives in the soil without light. However, whether *M. incognita* possesses phototaxis or pigments remains unknown.

Here, we showed that, despite the lack of specialized light-sensing organs in the head or other parts of the body, *M. incognita* exhibits negative phototactic behavior that is efficiently induced by shorter wavelength of light. This behavior seems to be important for survival to avoid toxic ultraviolet light, and it may contribute to the nematode's ability to live in soil without light.

2. Materials and Methods

2.1 Nematode Preparation

M. incognita were prepared as in Nishiyama et al. (2015). Briefly, five or six tomato plants (cultivar Pritz; Kaneko Seeds Co., Ltd., Japan) were inoculated with approximately 120,000 *M. incognita* J2 in soil. The nematodes were isolated from Koshi, Kumamoto, Japan, and maintained in the NARO Kyushu Okinawa Agricultural Research Center. Two weeks after the inoculation, the tomatoes were moved to a hydroponic culture system, and nematodes were collected from the liquid medium.

2.2 Long-Term Phototaxis Assay on Agar Plates

1.5% agar medium without any nutrients was prepared in plastic petri dishes 6 cm in diameter (TPP Ltd., Japan). A total of 2,000 nematodes were placed in the center of an agar plate and left for 12 h at room temperature with a black paper shade (Figure 1A). Agar plates were irradiated by white light (fluorescent lamp, EFD15EN/13), blue light (470 nm LED, IS-mini, CCS, Japan), and red light (660 nm LED, IS-mini, CCS, Japan) from one side (Figure 1A). The distribution of nematodes on each agar plate was observed and photographed by a GT-F600 scanner (Epson). Binarization of nematode distributions was performed using Image J v1.46r (NIH, USA) (Figure 1B). Three strips, each 3 cm wide, were appointed in the binarized photograph, and integration of the nematode area was calculated by Image J (Figure 1B). An average of the integrated data for the three strips was calculated by Microsoft Excel, version 15.16.

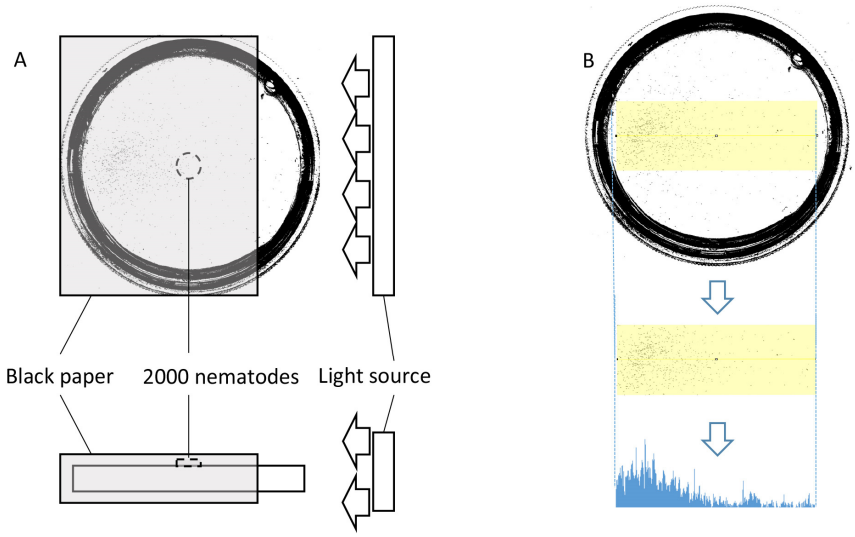

Figure 1. Methods for evaluating nematode phototaxis. A: Nematodes were placed in the center of a 1.5% agar plate (dotted circle) and left for 12 h with 80% of the plate shaded by black paper. Light sources were placed 5 cm away from the plate. B: Calculation of nematode distribution. The photographs were binarized by Image J. A stripe area (3 cm wide; first arrow) was sampled from the nematode plate. The black dotted area was considered the nematode area and was integrated at each point from left to right (second arrow)

2.3 Short Term Phototaxis Assay Using Short-Wavelength Light

Nematodes were irradiated by ultraviolet (365 nm: filter set 49, Zeiss), blue (470 nm: filter set 38HE, Zeiss), blue-green (500 nm: filter set 46, Zeiss), and green (550 nm: filter set 43HE, Zeiss) lights on a glass slide, observed using an upright microscope (Imager M1, Zeiss, Germany), and photographed in animation using a camera (DP71, Olympus). Light avoidance duration was measured using a soft, DP 3.2, Olympus.

3. Results

Long-term phototaxis assay on agar plates was performed using *M. incognita* by irradiating white, blue and red light. Most of all nematodes were observed on the side of the agar plate opposite to the white-light irradiation (Figure 2A). The nematode density was higher on the side opposite the light source. We further examined blue light (short-wavelength light) and red light (long-wavelength light). We found that blue light triggered negative phototaxis more efficiently than red light in nematodes (Figure 2B, C).

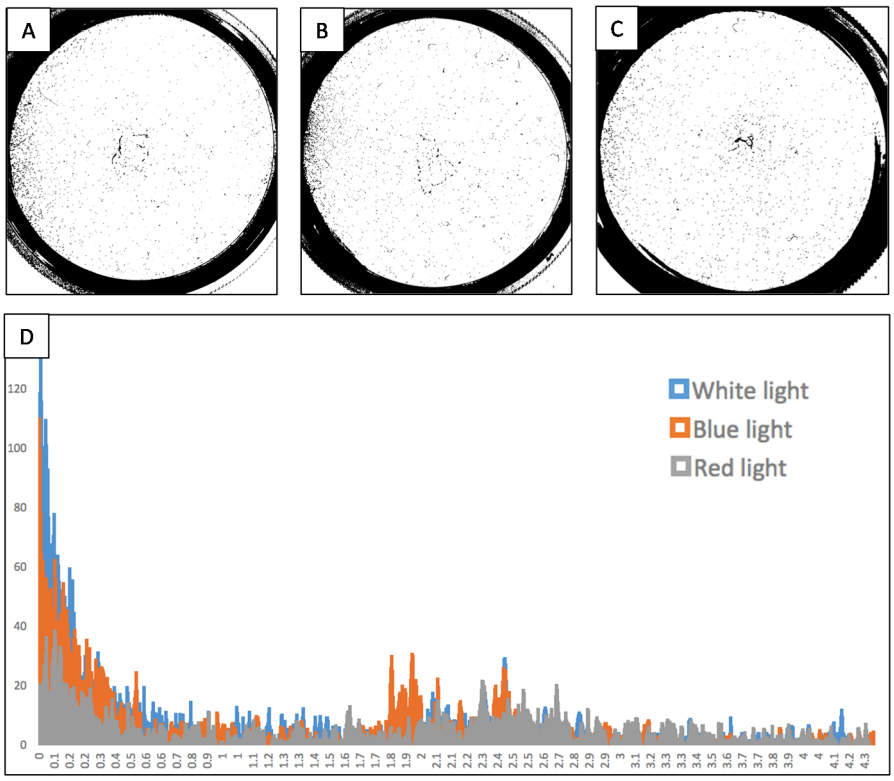

Figure 2. Long term phototaxis assay of *M. incognita*. Distribution of nematodes irradiated by white light (A), blue light (B), and red light (C) (n=3). D: Nematode distributions expressed as integrations of the black areas. The x-axis gives the distance (cm) from the left side of the stripe as shown in Figure 1B. The y-axis shows the integration area of the black pixels

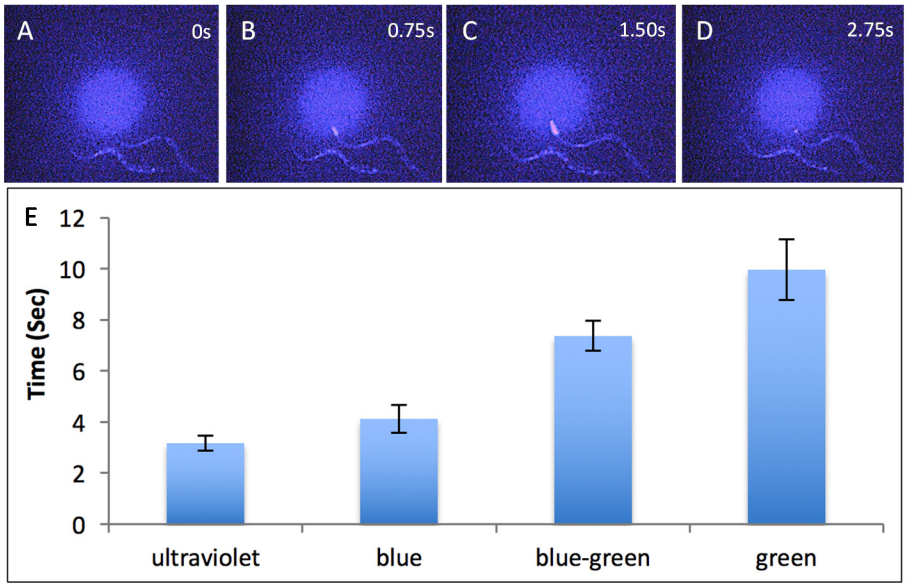

Figure 3. Short-term phototaxis assay of *M. incognita*. Snapshot images showing ultraviolet light avoidance responses in nematodes moving forward (A–D). Light was delivered via the objective to the head of a worm moving forward under the microscope. In this case, the nematode responded by stopping forward movement at 1.5 s and initiating reversal, leaving the light irradiation area at 2.75 s. E: The average time for head avoidance responses for ultraviolet, blue, blue-green, and green light. The data are the means with the error bars indicating the standard deviations (n = 42)

The negative phototaxis results were also expressed as a histogram. The shaded area of nematodes in the 3-cm strips on the agar plate was integrated, as shown in Figure 1B. The nematodes were significantly distributed toward the side opposite the white- and blue-light irradiation. Red light led to a limited negative phototactic response (Figure 2D). These results indicate that *M. incognita* negatively responds to white and blue light more than to red light.

Because blue light was more effective than red light at inducing a response (Figure 2B-D), the short-term response of nematodes to shorter wavelength of light was examined (Figure 3). Nematodes were independently irradiated by near ultraviolet (365 nm), blue (470 nm), blue-green (500 nm), or green (550 nm) light via a microscope objective lens. When the nematodes recognized ultraviolet light in the region of the head (Figure 3A-C), they reversed direction and moved away from the light region (Figure 3D). We measured the duration of this response and found that nematodes escaped from the light spot in 3.1, 4.1, 7.4, and 10 seconds when irradiated by ultraviolet, blue, blue-green, or green light, respectively (Figure 3E). These results indicate that nematodes avoid shorter wavelengths and that they are able to respond within a few seconds after light irradiation.

4. Discussion

Here, we found that *M. incognita* exhibits negative phototaxis. These nematodes efficiently responded to short-wavelength light. It has been reported that light stimuli elicits negative phototaxis in *C. elegans,* which lives in a dark environment like *M. incognita* (Ward et al., 2008). *M. incognita* may respond by returning to a dark environment like *C. elegans* when exposed to light. *M. incognita* individuals responded when their heads came under the light spot, indicating that they recognize light at the head. This negative phototactic behavior may be important for keeping the worms in a dark environment.

Ultraviolet light was the most effective for eliciting negative phototaxis in *M. incognita*. Ultraviolet light is thought to be more efficient at paralyzing worms than violet or blue light (Ward et al., 2008). The fruit fly, *Drosophila melanogaster,* has compound eye, and exhibits a negative phototaxis to ultraviolet light (Harris et al., 1976). Thus, it seems that the light avoidance response is generally essential for survival in insects.

Light signals are transduced by the activity of transient receptor potential (TRP) family channels in Drosophila (Wang and Montell, 2007). However, *C. elegans* and vertebrates utilize cyclic nucleotide-gate (CNG) channels (Fu and Yau, 2007; Ward et al., 2008). So, we searched homologues of *C. elegans* TAX-2 (NP_492427, NCBI) and the Drosophila TRP (NP_476768, NCBI) protein sequences using expressed sequence tag (EST) database of TBLASTN program (Altschul, 1997) at *Meloidogyne* genomic resources (http://meloidogyne.inra.fr/genomic_resources). Two ESTs of *M. incognita*, ra99h10 and rbd06 were identified to have significant homology with TAX-2, producing E-values of 1.5e-57 and 2e-36, respectively. However, no TRP homologs could be identified (data not shown). This suggests that *M. incognita* may have a mechanism similar to that of *C. elegans* for transducing light signals. Furthermore, *M. incognita* may utilize a group of ciliary sensory neurons for phototaxis, as is used in *C. elegans* (Ward et al., 2008). The two nematodes seem to have phototransduction cascades mediated by CNG channels, similar to those of vertebrates. It may be that the two soil-dwelling nematodes, *M. incognita* and *C. elegans,* have similar mechanisms for maintaining their position in the soil and that the nematodes inherited the CNG mechanism from their common ancestor. The CNG pathway may be an ancestral mechanism in the light-signaling pathway. In this case, urbilaterians, the ancestral group to vertebrates and insects (Adoutte et al., 1999), should have also possessed the CNG pathway.

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