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Fluorescence of the "fire-chaser" beetle *Melanophila acuminata*

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This paper is dedicated in memory of Jerry Wolken

Abstract

Melanophila acuminata beetles are attracted to forest fires over long distances by a pair of specialized infrared sensory organs. To date, there is no knowledge of their ability to detect or emit fluorescent radiation. We studied the *Melanophila acuminata* infrared sensory organs histologically and by using fluorescent microscopy, acoustic–optic tunable filter microscopy, and two-photon microscopy to identify fluorescence. We found fluorescent absorption at radiation wavelengths of 480 nm and emission at 570 nm. The functional role of this novel fluorescence is, as of yet, unknown but may be applied to species classification, identification and behavioral studies.

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Keywords: Melanophila acuminata; Beetle; Fluorescence; Fire; Thermoluminescence

1. Introduction

Melanophila acuminata beetles are rarely found in forests under normal conditions but are attracted to forest fires in large numbers [1]. These beetles travel distances of over 20 km to the fires to mate and lay eggs in burnt conifer trees [2,3]. They respond to the fires via a pair of specialized sensory organs located next to their mesothoracic legs. Each sensory organ or pit is 150 μm in diameter and contains of 50–100 sensilla; each sensillum is accompanied by a wax gland and has a cuticular lens sphere with a diameter of 15 μm [4]. Evans [5] successfully determined with behavioral experiments that the beetles respond and change directions according to the infrared source. Furthermore, he was able to rule out that the detection of fires occurs via olfactory or auditory cues.

Phylogeny and classification of specific species depends, in part, on unique features. *M. acuminata* detect infrared radiation from fires at wavelengths of 3 and 10–25 μm [3]. The response of the sensory organs to other wavelengths is unknown. It was our purpose to explore the fluorescent activity of the sensory organs and determine whether they contain fluorophores. Fluorophores emit light in response to exposure to radiation from an external source and are classified and described according to their absorption and fluorescence properties, including their spectral profiles, wavelengths of maximum absorptions and emission, and the fluorescence intensity of the emitted light.

2. Materials and methods

Adult *M. acuminata* were collected by entomologists Richard Westcott (Oregon Department of Agriculture, Salem) and Nathan Schiff (US Forest Service, Stoneville, MS) from two recent forest fire burn locations: in the Sandy River delta, Multnomah Co., Oregon, 21 m elevation and Olallie Lake, Jefferson Co., Oregon, in the Cascade Range at 1615 m elevation. The beetles were kept at 25 °C and were fed raisins, peanuts and water in a humidified environment. The beetles were immediately euthanized prior to specific investigations, or kept alive where noted.

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2.1. Histology

Frozen sections of sensory pits were mounted on glass microscope slides and triple stained [6] for routine microscopy to determine histological detail.

2.2. Confocal fluorescent microscopy

In vivo assessment of fluorescence of the beetles' sensory pits was undertaken by holding the beetles in clay during the time to exposure. A fluorescent Nikon confocal microscope was used. For excitation, a multi-argon laser (40 mW; wavelengths: 458, 488 and 514 nm) and a helium-neon (red) laser (10 mW; wavelength 633 nm) were used. Average, low-pass and high-pass filters were utilized and a cutoff frequency slider was used to vary the range of the frequencies that were passed by the filter. A monochrome CCD camera (Hamamatsu Digital Camera CA 742-95) was employed to image fluorescent emission.

2.3. Spectrophotometry

Dissected sensory pits were placed into cuvettes and analyzed using a temperature-controlled Beckman DU 7400 UV/Visible diode array spectrophotometer. Specimens were scanned over a wavelength range from 340 to 520 nm and absorption was determined and graphed.

2.4. Acoustic–optic tunable filter (AOTF) microscopy

The AOTF microscope (Chromodynamics Inc, Pittsburgh; Nikon) [7,8] was used to determine the fluorescence spectrum of the entire sensory pit by modulating the wavelength and amplitude of illuminating laser light. An excitation spectrum from 400 to 675 nm was employed. A CCD camera (Hamamatsu Digital Camera CA 742-95) was utilized to ascertain fluorescent emission.

2.5. Laser microdissection and two-photon microscopy

M. acuminata were prepared for the further analysis using two-photon microcopy (Beckman Institute, CA). The sensory organs were embedded in OCT compound and frozen at $-80\,^{\circ}C$ for cryostat sectioning followed by 2% Gimsa nucleic acid stain. Sections were desiccated under vacuum for 10 min, and placed under a stereoscope. A drop of water was applied to remove the OCT mounting compound. Sensory organs were then microdissected using Beckman Laser Institute laser scissor/tweezers system and the optical characteristics of the dissected components were determined. Two-photon microscopy was performed on dissected sections.

2.6. Two-photon imaging and spectroscopy system

Two-photon excitation of fluorescence allowed the use of a longer wavelength of light (between 690 and 800 nm) compared to single photon confocal microscopy. The nonlinear optical absorption property of two-photon excitation limited the fluorochrome excitation to the point of focus. The two-photon imaging system was as previously described [10] with the addition of a Spectra-Pro 150 spectrograph with a 300 grooves/mm grating blazed at 500 nm (Acton Research Corporation), equipped with a high dynamic range MicroMax: 512 BFT CCD camera (Princeton Instruments). A spectrograph was attached to the side port of the microscope. Subsequent to single scan image acquisition, the emission spectrum from the same optical section was collected at a 10-s acquisition time (1 scan/acquisition). The two-photon laser scanning fluorescence microscope system consists of a 5 W Verdi laser (Coherent, Santa Clara, CA) that is used to pump a titanium:sapphire ($Ti:Al_2O_3$) laser (Mira 900F, Coherent). In order to obtain the low average powers required to maintain tissue health at the sample, a half wave plate and polarizer were placed in the input beam path to decrease the average power to 5–10 mW. The pulse train exiting the $Ti:Al_2O_3$ laser was deflected into the back port of a Zeiss Axiovert S100 2TV microscope using a PC controlled galvanometer driven X–Y scanner (Series 603X, Cambridge Technology, Inc., Watertown, MA). Two-dimensional (x–y plane) images (256×256 pixels) were acquired from various depths (z) into the sample at a rate of 1 frame/s (pixel dwell time of 16 ms/pixel) with a single-photon counting PMT (R7400P, Hamamatsu Corp., Bridgewater, NJ), covering an area of $35 \times 35\,mm^2$ for the $63\times$ microscope objective. Resolution was approximately 0.4 and 1 mm in the x–y and z image planes, respectively. Using this configuration, we routinely obtained tomographic images to depths of 1–1.2 mm.

3. Results

3.1. Histology

Histological sections confirmed previously detailed studies and were used to correlate structure to fluorescent regions. Each sensory organ or pit contained 50–100 sensilla. Each sensillum had a diameter of 15 μm and was composed of an outer shell (epicuticle) and an inner sphere that consisted of three distinct regions: a lipid region; a protein region that was 100 nm wide and ~7.0 μm thick; a central lipid-protein region; and a polysaccharide band around a single neuron (Fig. 1).

3.2. Confocal fluorescent microscopy

Confocal miscroscopy was employed as a screen to determine fluorescent activity. Using a monochrome camera, confocal microscope, and a laser at 488 nm, fluorescent activity of the *M. acuminata* sensory pit was encountered in vitro with an absorption peak at approximately 460 nm and an emission of 570 nm, a photograph of which is shown in Fig. 2. With the use of different filters,