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ORIGINAL ARTICLE

Aposematic potential of ultraviolet-visible blue fluorescence in larvae of a cyanogenic zygaeniid moth *Eterusia aedea* (Lepidoptera: Zygaenidae)

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Abstract. Some insects deter predators by sequestering toxic compounds and displaying aposematic coloration. The subfamily Chalcosiinae (Lepidoptera: Zygaenidae) is known for the vivid larval coloration and cyanogenic glycoside secretion by larvae and adults. However, the larvae of a Chalcosiinae moth, *Eterusia aedea,* exhibit a subdued reddish-brown appearance, which does not visually signal toxicity. In this study, we report that *E. aedea* larvae and their secreted mucus emit strong blue fluorescence under ultraviolet (UV) light. Fluorescence analysis of the mucus revealed a peak emission at 446.0 nm, a purplish-blue wavelength. Given that avian and reptilian predators possess UV-sensitive vision, this fluorescence may serve as an aposematic signal. While the ecological function of fluorescence in insects remains unclear, our findings suggest that larval fluorescence in *E. aedea* may play a role in predator deterrence. Further studies are needed to determine whether this fluorescence is perceived and learned by natural predators as a warning signal.

INTRODUCTION

Some insects avoid being preyed upon by predators by secreting fluids containing poisons when attacked by predators, thereby making the predators have an unpleasant experience. These insects often have showy external appearances as an aposematic signal to make predators learn the combination of the unpleasant experience caused by the poison and their appearance (Ruxton et al., 2018).

The subfamily Chalcosiinae (Lepidoptera: Zygaenidae) is a group of moths that includes around 70 genera and 370–400 species (Yen et al., 2005), distributed in an area ranging from Palearctic eastern Asia, through subtropical south-east Asia, to the Melanesian and Micronesian archipelagos. Many of these species are thought to sequester cyanogenic glycosides in their bodies through uptake from host plants or by de novo biosynthesis (Nishida, 2002; Yen et al., 2005).

Eterusia aedea (Linnaeus) is a moth belonging to Chalcosiinae that is distributed from the Indian subcontinent including Sri Lanka through Indochina, Tibet, China, and Taiwan to most island groups of Japan (Yen, 2004). In Japan, this species mainly uses Theaceae and Pentaphylacaceae plants as host plants (Owada, 2013), and in India, it is considered an important pest of tea plants (Roy et al., 2018). The larvae of this species secrete a clear mucus containing cyanogenic glycosides through pores on the verrucae on their body surface (Naumann & Feist, 1987; Nishida, 2002; Yen, 2004; Yen et al., 2005).

Most larvae of Chalcosiinae species wear gaudy maculations of yellow, red, black, and silver, which are thought to be aposematic signals of toxicity. However, the larvae of *E. aedea* have a monotonous and subdued reddish-brown coloration that is unlikely to function as an aposematic signal (Fig. 1a). We report that these larvae exhibit a vivid blue fluorescence under UV LED light, with its toxic mucus glowing even more intensely.

MATERIAL AND METHODS

Insects

Mated females of *Eterusia aedea* were captured at multiple coppices on Ishigaki Island, Okinawa Prefecture, Japan (24.4°N, 124.2°E) in September 2018. These were reared to establish a cumulative line for this study. Initially, the captured female moth was placed in a clean plastic cup (129 mm diameter × 97 mm height, Biocup® 129∅860B/129∅FSL, Risupack Co., Gifu, Japan) under laboratory conditions (25 ± 2°C, 14L : 10D), with

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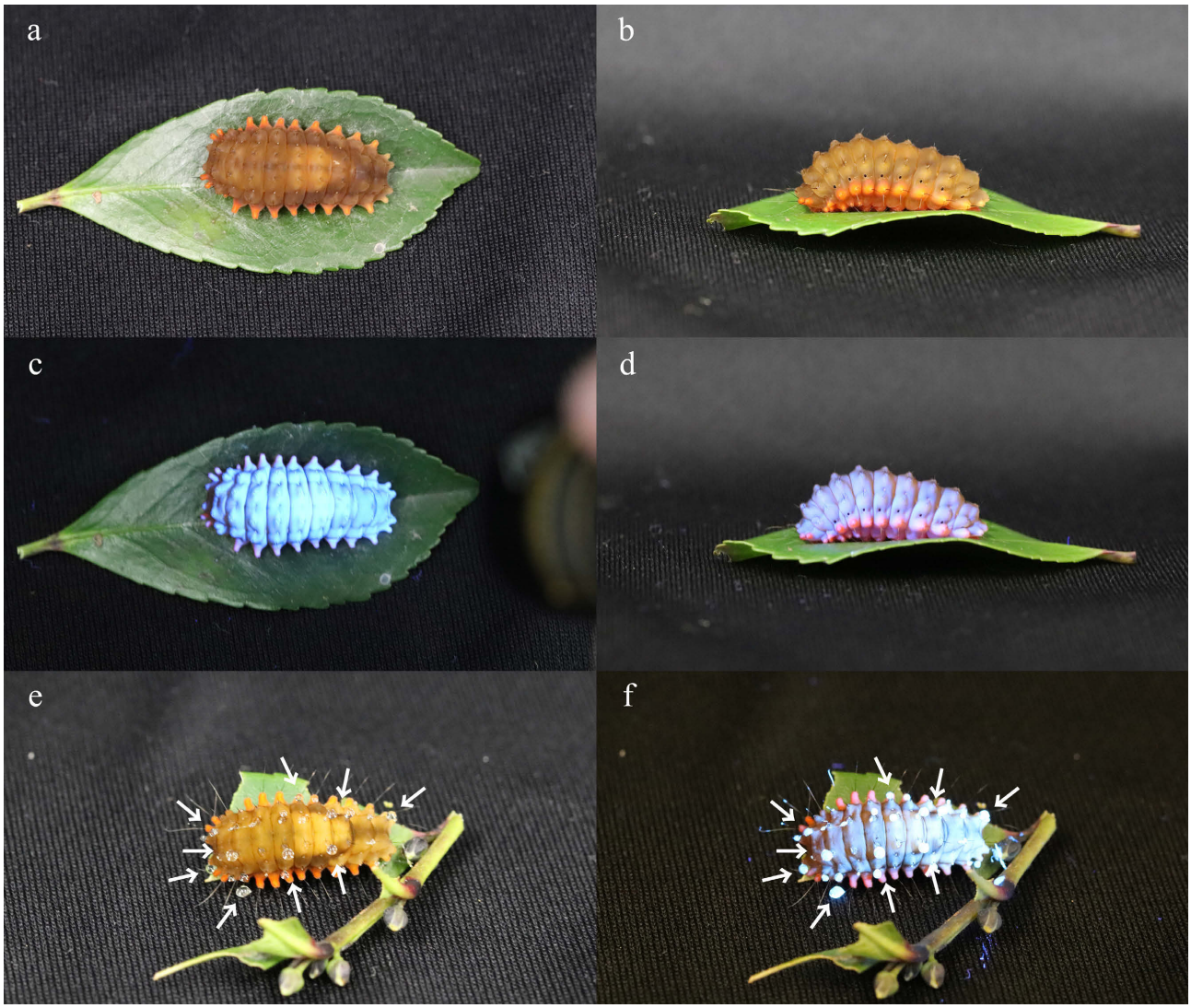

Fig. 1. Last instar larva of *Eterusia aedea*. a, b, e: white light (a – dorsal view, b – lateral view, e – dorsal view with mucus drops); c, d, f: UV light (c – dorsal view, d – lateral view, f – dorsal view with mucus drops). Arrows on Fig. 1e and 1f indicate the mucus drops.

some fresh leaves of *Eurya japonica* Thunb. to lay eggs. The female did not lay eggs on the plants, but preferred to lay them in the gaps in the aluminum foil that wrapped the cut ends of the plants. The hatched larvae were fed on fresh leaves of *E. japonica* in the same plastic cups the adults were reared; same feeding plant was used to feed the first four instars. From the fifth instar onwards, larvae were fed with fresh leaves of *Camellia japonica* L., which are planted in large numbers on the campus of Tottori University (35.29°N, 134.11°E). The emerging adults were sexed based on the shape of their antennae. The adults matured sexually about one week after emerging. The sexually mature females and males were paired using the hand-pairing method and used for egg collection to obtain the next generation. The adults were supplied ad libitum with the non-alcoholic beverage, Pocari Sweat® (Ohtsuka Pharmaceuticals Co., Tokyo).

Observing and photographing fluorescence

A UV LED light (365 nm, 10 W; Alonefire SV003, Shenzhen Shiwang Technology, Shenzhen, China) was shone from a distance of 30 cm from the last instar larva, and the photographs were taken with a single-lens reflex camera (body: EOS 9000D, Canon, Tokyo, Japan; lens: SP 90 mm F/2.8 Di MACRO 1:1 VC USD Model F017, Tamron, Saitama, Japan) (Fig. 1). Larvae exposed to visible light only and visible light + UV light were photographed under the following conditions: subject-lens distance 40 cm, aperture F/5.6, shutter speed 1/60 s, ISO speed 6400/39°. To confirm whether other species belonging to the same family emit UV-excited fluorescence similar to *E. aedea*, final instar larvae of *Hedina consimilis* (Leech) (Zygaenidae: Procridinae), colored with vivid aposematic patterns, collected from the campus of Tottori University were photographed under the conditions above.

Mucus collection and fluorescence analysis

To collect the clear mucus secreted by *E. aedea* larvae through pores on the verrucae on their dorsal body surface, each larva was stimulated on the back using a glass rod (Glass rod, 1500 mm, ∅3, AS ONE, Osaka, Japan). The mucus was wiped off with the glass rod and transferred to the inner wall of a glass vial (Mighty Vial No. 6, clear 28 mL, Maruemu, Osaka, Japan). This procedure was repeated with 10 larvae. After collection, 10 mL of either ultrapure water, 99.5% ethanol, or *n*-hexane was added to the vial to dissolve the mucus. The resulting extract was stored at –20°C until fluorescence analysis. UV light was then applied to measure the excitation and fluorescence wavelengths of the extract.

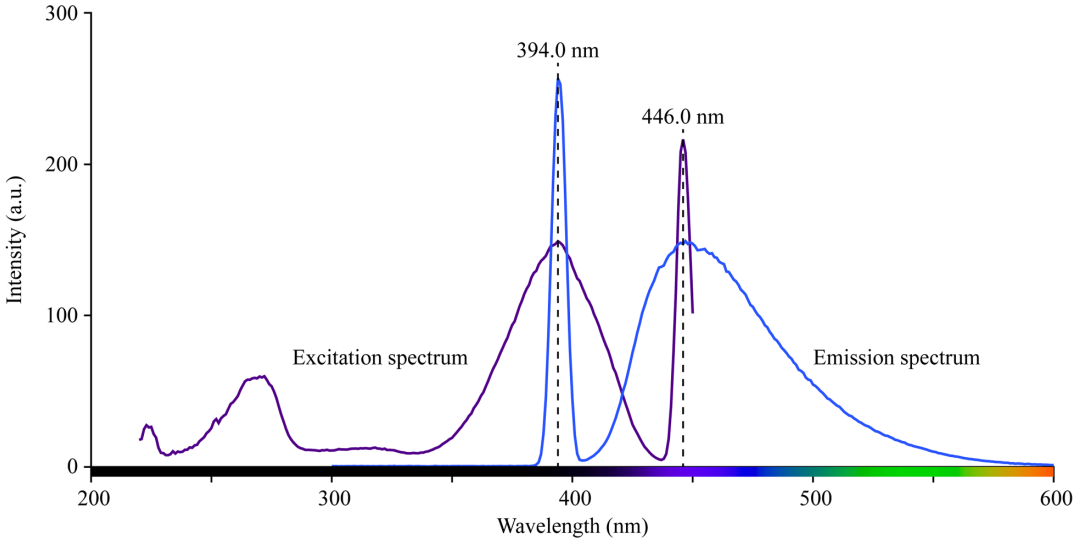

Fig. 2. Excitation and emission spectra of fluorescence from the mucus of *Eterusia aedea* larvae. Violet line: excitation spectrum, blue line: emission spectrum.

Before analysis, the vials containing the extracts were exposed to UV LED light (Alonefire SV003) to confirm that the extracts fluoresced. As the fluorescence of the extract was too strong to be tested using fluorescence analysis, the concentration of the extract was diluted 1,000× using the same solvent as that used to dissolve the mucus. The fluorescence from the extract was measured with a fluorescence spectrophotometer (RF-5300PC, Shimadzu, Kyoto, Japan) using a 4-sided quartz cell at 25°C. The excitation spectra were scanned from 220.0 to 450.0 nm at a fixed fluorescence wavelength of 446.0 nm, and the emission spectra were scanned from 300.0 to 600.0 nm at a fixed excitation wavelength of 394.0 nm. The excitation and emission slits were 5.0 nm, the scanning rate was 1,000 nm/min, and the resolution was 1.0 nm.

RESULTS

Observation and imaging of fluorescence

When *E. aedea* larvae were exposed to 365 nm UV LED light, their body surfaces emitted a strong blue fluorescence (Fig. 1). The fluorescence on the lateral regions, which appeared slightly redder than the body color, was weaker than that of the rest of the body, which exhibited intense fluorescence. When illuminated from above, the larval body surface glowed more brightly than when exposed to UV light from the side (Fig. 1c, 1d). Under UV illumination, both the larval body surface and the mucus secreted by the larvae fluoresced in the same color tone (Fig. 1a, 1e). However, the fluorescence of the mucus was more intense than that of the body surface. Therefore, we used the mucus for fluorescence analysis. *Hedina consimilis* larvae did not emit UV-excited fluorescence.

Mucus collection and fluorescence analysis

Mucus collected from the larvae was tested for solubility in ultrapure water, 99.5% ethanol, and *n*-hexane, and the mucus was dissolved only in 99.5% ethanol. The excitation and fluorescence emission spectra of the mucus were measured using a spectrofluorometer. When excited at 394.0 nm, the fluorescence emission spectrum exhibited a prominent peak at 446.0 nm (Fig. 2).

DISCUSSION

When exposed to UV light, the larvae of *Eterusia aedea* and their toxic mucus emitted intense fluorescence (Fig. 1). The fluorescence was stronger in the mucus than on the larval body surface, with the mucus displaying a peak emission wavelength at 446.0 nm, corresponding to a purplish-blue color (Fig. 2). To our knowledge, this is the first report of fluorescence emission in Zygaenidae moths.

Recent studies have documented UV-induced fluorescence in the larvae of various lepidopteran species (Moskowitz, 2017, 2018; Sourakov, 2017, 2019; Messenger et al., 2019; Anselmo et al., 2024). Although Zygaenidae species do not exhibit UV-induced fluorescence on their body surface, several species of Limacodidae, which belong to the same superfamily Zygaenoidea as Zygaenidae and are covered with tubercles with urticating hairs and aposematic color patterns, exhibit fluorescence on all or part of their bodies. Many of these studies report that fluorescence excited by UV light helps to locate lepidopteran larvae at night. On the other hand, Sourakov (2017, 2019) revealed that in several species of larvae known to be toxic and exhibiting various aposematic color patterns, only part of the markings fluoresce, suggesting that this fluorescence may also be a type of aposematic signal. However, until we figure out what predators can see this fluorescence and if they recognize it as an aposematic signal, the ecological significance of this fluorescence remains unclear.

Humans, with their trichromatic vision, have limited sensitivity to short-wavelength blue light due to the collagen nanostructure of the cornea (Smith & Pokorny, 1972; Tsukahara et al., 2010). As a result, even under direct sunlight, the fluorescence emitted by *E. aedea* larvae is barely perceptible to the human eye, making the larvae appear as plain reddish-brown caterpillars. In contrast, many avian and reptilian predators of lepidopteran larvae possess either trichromatic or tetrachromatic vision, enabling them to detect UV wavelengths (Osorio et al., 1999; Loew et al., 2002; Hart & Hunt, 2007; Fleishman 2024). This suggests

that the fluorescence of *E. aedea* larvae may be fully visible to these predators and integrated into their perception of the larval body color.

Both larvae and adults of this species are believed to sequester cyanogenic glycosides as a chemical defense (Nishida, 2002; Yen et al., 2005). The adults exhibit conspicuous warning coloration, with a yellow abdomen and fore- and hindwings featuring a striking green-to-lapis lazuli ground color with white spots. In contrast, the larvae appear monotonous and plain reddish-brown, a coloration that does not resemble typical aposematic signaling to the human eye. Given that the larvae are chemically defended, their lack of obvious warning coloration seems paradoxical. If the fluorescence observed in this study serves as an aposematic signal for birds and reptiles, these predators may recognize the larvae as possessing a warning coloration similar to that of the adults. In Chalcosiinae species closely related to *E. aedea*, many of the larvae exhibit aposematic color patterns that are as vivid as those of the adults (Yen et al., 2005). The fact that only a few species, including *E. aedea*, have uniformly dull body colors suggests that these species have lost their aposematic color patterns during evolution for some reason. This evolution may represent a double strategy, where aposematic signals are displayed to birds and reptiles while also serving as cryptic coloration for species with reduced sensitivity to blue or ultraviolet light.

The potential role of UV-induced coloration in aposematism has been debated for some time (Lyytinen et al., 2001, 2004; Yeager & Barnett 2020, 2021; Stella & Kleisner, 2022). However, relatively few studies have investigated the ecological function of fluorescence emitted by prey species. In fireflies, bioluminescence has been suggested to deter bat predation (Leavell et al., 2018). Yet, UV-induced fluorescence present in some firefly species does not appear to function as an aposematic signal (Wilcox & Lewis, 2019). Similarly, while many scorpions exhibit fluorescence, their role in predator deterrence remains unverified (Gaffin et al., 2012).

To determine whether the fluorescence of *E. aedea* larvae serves an aposematic function, several key questions must be addressed. First, what are the primary predators of these larvae in the wild? Second, do predators that have had aversive experiences with the larvae associate their coloration with unpalatability? Third, is fluorescence a factor in this learned avoidance? Additionally, it is important to investigate whether other zygaeniid larvae with conspicuous warning coloration also exhibit fluorescence. The larvae of *H. consimilis* had no UV-excited fluorescence, but the presence or absence of UV-excited fluorescence in the larvae of other Chalcosinae species, which are more closely related to *E. aedea*, should be further investigated. Further studies are needed to investigate the ecological role of fluorescence in Zygaenidae species, and to explore whether the fluorescent signal is indeed a reliable aposematic signal recognized by their predators.

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