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# Spectacular Fluorescence Emission in Sea Urchin Larvae

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**ABSTRACT**—Strong fluorescence emission occurred in sea urchin larvae when irradiated with blue light under a fluorescence microscope. The blue light irradiation first broke red granules in the pigment cells, releasing green fluorescent substance(s) into the cytoplasm of the pigment cells. The released and dispersed fluorescent substance(s) then made the entire pigment cell emit strong green fluorescence. With prolonged blue light irradiation, the pigment cell itself bursted, dispersing the fluorescent substance(s) into the body cavity or seawater. This resulted in “explosive emission” of the fluorescence. Cells that can emit such fluorescence first appeared at the late-blastula stage, proliferated with development, and changed into the red-pigmented cells. Similar fluorescence emission was observed in the larvae of *Clypeaster japonicus*, *Hemicentrotus pulcherrimus*, *Anthocidaris crassispina* and *Pseudocentrotus depressus*, of which *C. japonicus* larvae displayed the strongest fluorescence.

## INTRODUCTION

Organisms that can emit fluorescence were reported in various species from bacteria to fish (Haneda, 1985). In Echinodermata, strong fluorescence emission was reported with sea star, sea lily, and sea cucumber. However, no such examples have been reported in the class Echinoidea. The egg and embryo of many kinds of sea urchin are known to display auto-fluorescence, although it is usually weak. We were surprised to observe an unusually strong, explosive fluorescence emission in the pluteus larvae of a sea urchin, *Clypeaster japonicus*, upon irradiation with blue light under a fluorescence microscope. Here we described this novel discovery.

## MATERIALS AND METHODS

*Clypeaster japonicus*, *Hemicentrotus pulcherrimus*, *Anthocidaris crassispina* and *Pseudocentrotus depressus* were collected from the local coast of Toyama Bay and kept in running seawater aquaria at 13–25°C. Shedding of gametes was induced by injecting 0.5 M KCl into the coelomic cavity. Sperm were kept ‘dry’ in the refrigerator until use; eggs were shed into filtered seawater, and washed three times by sedimentation. Fluorescence emission was observed by an

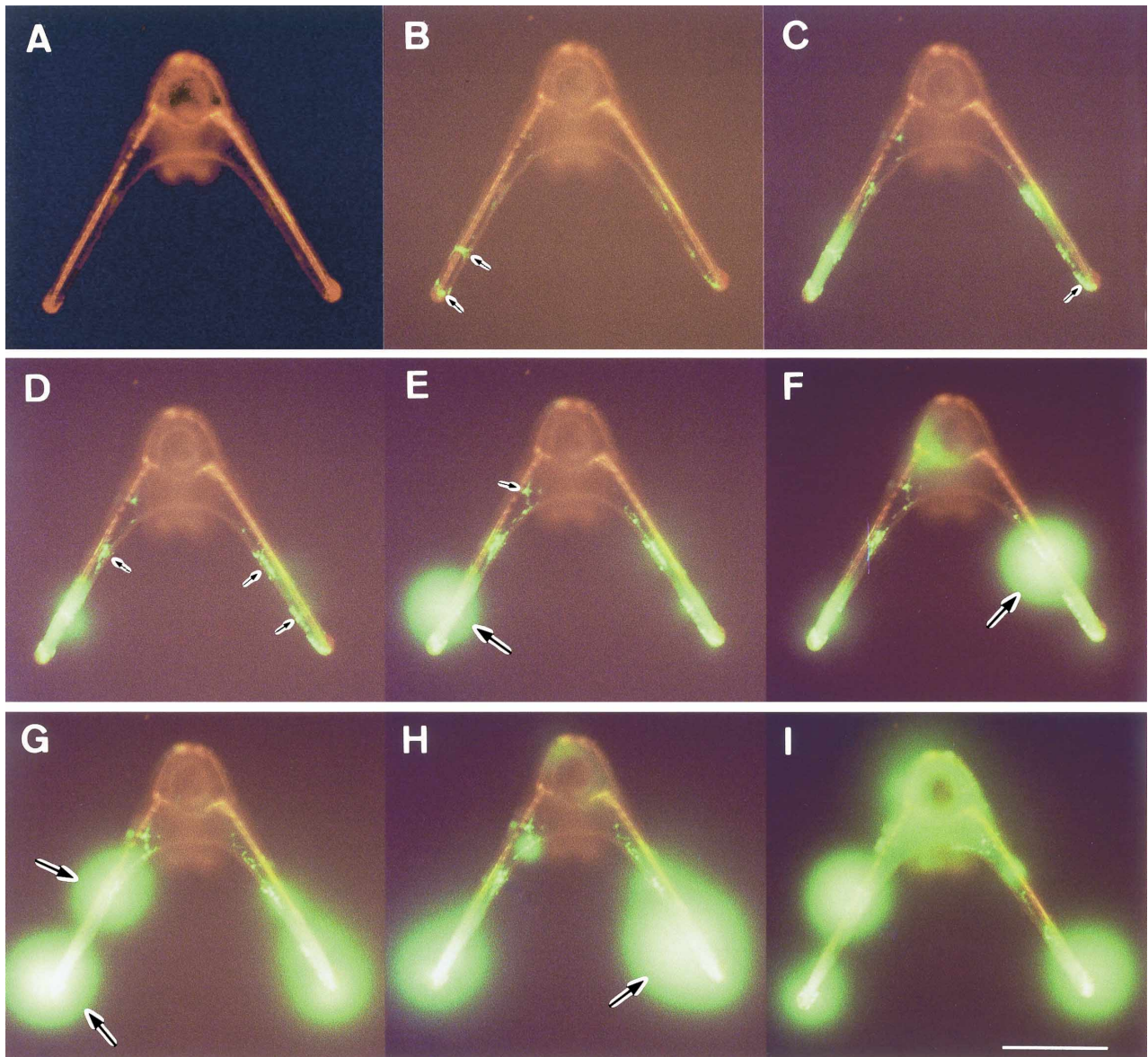
Olympus BH2 microscope equipped with epifluorescence optics. Micrographs were taken on Fujicolor Super HG 1600 film or Kodak Tri-X pan film. The emission was measured using a PM-10 microscope spectrometer (Hamamatsu Photonics K.K., Hamamatsu, Japan).

## RESULTS AND DISCUSSION

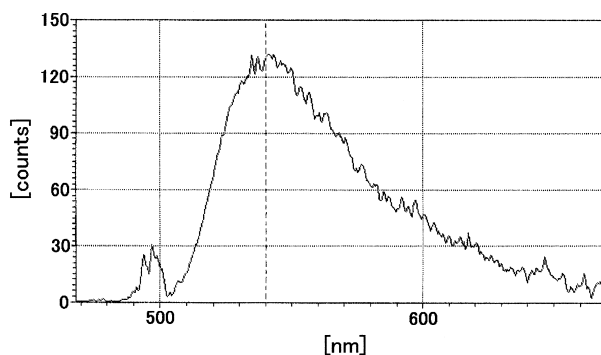
When we observed some pluteus larvae of the sea urchin, *C. japonicus*, under a fluorescence microscope, we found an unusually strong, explosive fluorescence emission (Fig. 1). Under the B excitation mode (450–490 nm), the emission had a maximum at about 540 nm (Fig. 2). About 1–2 minutes after the onset of excitation in our system, sporadic “explosive emission” of strong green fluorescence occurred at various parts on the body surface and the tips of the postoral arms (Figs. 1B,C). The fluorescence emission in one explosion lasted for a few seconds, repeating several hundred times over a period of >30 min. The emission was observed at the highest frequency at the tips of the postoral arms. Similar but somewhat weaker fluorescence emission was observed in the larvae of *Hemicentrotus pulcherrimus*, *Anthocidaris crassispina* and *Pseudocentrotus depressus*. In adult *C. japonicus* also, fluorescence emission occurred in the spines and the epidermis (data not shown).

The fluorescence was apparently emitted by red granules in the pigment cells. The change in the emission pattern

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**Fig. 1.** Fluorescence emission in a pluteus larva of *Clypeaster japonicus*. (A), before the onset of excitation at 450–490 nm (B-mode). Time after the onset of excitation in seconds: 60 (B), 80 (C), 90 (D), 100 (E), 110 (F), 120 (G), 130 (H), 180 (I). Epifluorescence illumination and dark-field illumination were used simultaneously. Small arrows show the pigment cells dispersed fluorescent substance(s) in its cytoplasm. When those pigment cells were broken down, each of them produces a spectacular halo of fluorescence (large arrows). Exposure, 1 sec. Bar, 100  $\mu$ m.

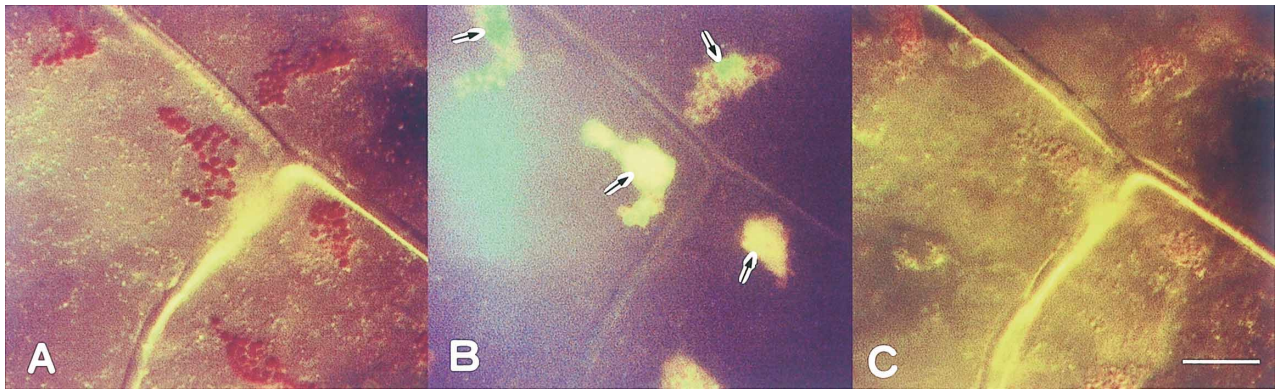


**Fig. 2.** A typical emission spectrum in a pluteus larva of *Clypeaster japonicus*. Excited under B (450–490 nm) excitation mode, and measured for 500 seconds.

suggests that blue light irradiation first disrupted the red granules, releasing and dispersing green fluorescent substance(s) into the cytoplasm of the pigment cell (Fig. 3B). The dispersed fluorescent substance(s) caused the entire pigment cell to emit strong green fluorescence (Figs. 1B, 3B). Prolonged blue light irradiation, then broke the pigment cell itself, causing the fluorescent substance(s) to disperse into the body cavity or seawater (Figs. 1C–1I). This results in “explosive emission” of the fluorescence. In *C. japonicus*, pigment cells were often broken down simultaneously with the breakdown of the red granules they contained, and then they rapidly emitted strong fluorescence. Each glow sphere of fluorescence seen in Figs. 1E–1I may possibly correspond to the breakdown of a single cell.

Developmentally, immature fluorescent pigment cells first



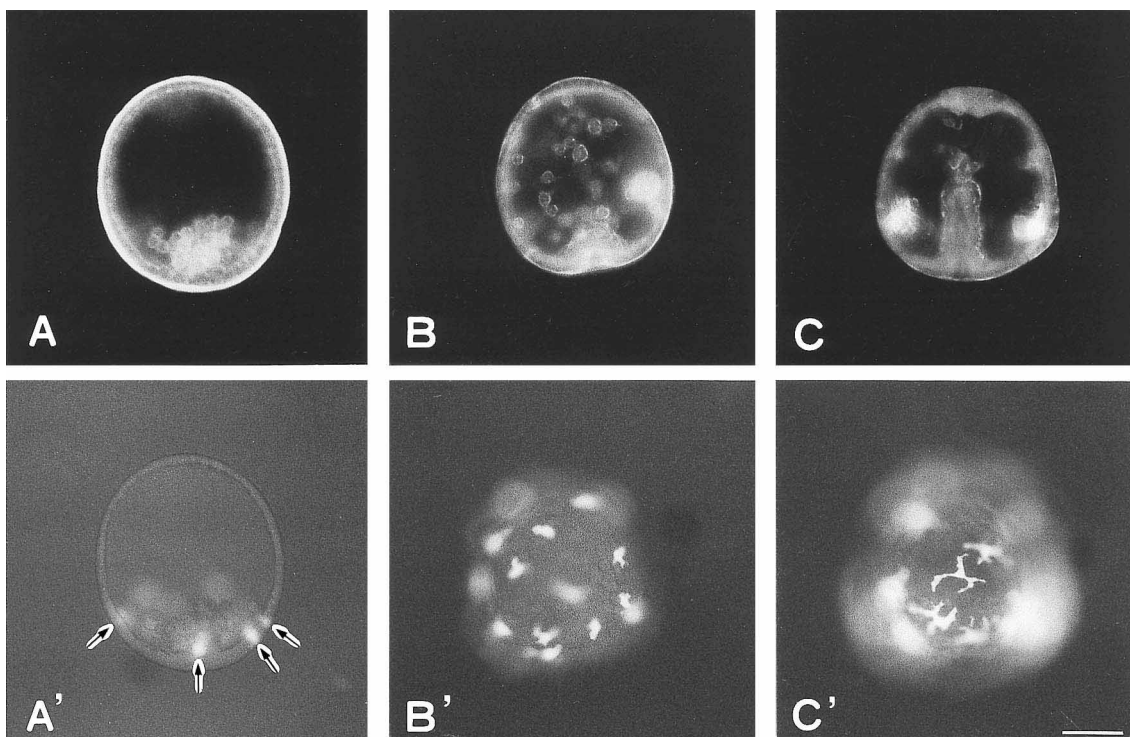


**Fig. 3.** The pattern of pigment granules breakdown in the pigment cells in a pluteus larvae of *Hemicentrotus pulcherrimus*. Before (A), 90 sec (B), and 180 sec (C) after the onset of excitation at B-mode. Epifluorescence illumination (B) and dark-field illumination (A, C) were used. Arrows show fluorescent substance(s) were released and dispersed into the cytoplasm of the pigment cells (B). Thereafter, we can recognize most pigment granules were broken (C). Bar, 10  $\mu$ m.

appeared in the vegetal basement of late blastula or early gastrula in *C. japonicus*. In an early stage, they apparently have no pigment granules and emitted only weak fluorescence (Fig. 4A'). These immature pigment cells increased the number (Fig. 4) and display amoeboid movements in the blastocoel and blastoderm, wherein they become mature pigment cells with well-developed pseudopodia. Those cells emitted bright fluorescence (Figs. 4B', C'). These observations suggest that the pigment cells identified by their fluorescence are

similar to those studied previously using a specific monoclonal antibody (Gibson and Burke, 1985).

The function of the pigment cells has not been established, although several studies have suggested that they may function in photoreception, myogenesis, or some nervous activity (Reyberg, 1979; Reyberg and Lundgren, 1979; Gras and Weber, 1977). In this study, we observed mature pigment cells have well-developed pseudopodia, and distributed on the tips of the postoral arms at high density. We therefore



**Fig. 4.** The appearance of fluorescence-emission cells in different developmental stages of *Clypeaster japonicus*. Cells that can emit weak fluorescence (arrows in A') appeared on the basal side of the vegetal ectoderm at the late-blastula (A, A'). They then proliferated and distributed between cells in the surface layer of whole embryo (B, B', C, C'). At the late-gastrula stage, they came to have well-developed pseudopodia and emit strong fluorescence (C'). A, B, C: dark-field micrographs; A', B', C': fluorescence micrographs. B' and C' were photographed focused on the surface. Bar, 50  $\mu$ m.

speculated that the pigment cells are involved in some nervous activity or morphogenesis.

To our knowledge, such a strong fluorescence as we found has not been reported before in sea urchin. The chemical and photochemical nature of the fluorescent pigment, as well as the mechanism by which blue light breaks open the pigment granules and pigment cells remains to be studied.

#### ACKNOWLEDGEMENTS

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