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Bioluminescence emissions of female fireflies of the species *Asymmetricata circumdata*

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Innumerable scientific studies have been carried out for over a century on the enchanting light of different species of fireflies. Almost all those studies have been performed on male specimens of fireflies; there has been no detailed study on bioluminescence emissions from females. Here we present a study on the light of female specimens of the Indian species of firefly *Asymmetricata circumdata* (Motschulsky). Steady-state spectra recorded on a high-resolution spectrometer show the peak wavelength at 565 nm, which is different from the one recorded for male specimens of the firefly-species. Time-resolved spectra show the flashes to be composed of three pulses. Diffraction pattern of this light in a grating shows the central principal maximum to be of color yellow which narrows down considerably in the first and second orders, while green and red color-sectors appear in the first order and become wider in the second one.

Keywords: Bioluminescence emissions, Fireflies, Diffraction Pattern

1 Introduction

The light of the firefly is the outcome of a very efficient reaction, called chemiluminescence reaction. Briefly, the reaction is as follows. The biochemical trigger O_2 excites the luciferin molecule to the oxyluciferin state in presence of the catalyst luciferase and Mg-ATP. Visible light is emitted when oxyluciferin decays to its ground state. It has been shown that the light of the firefly is the manifestation of an oscillating chemical reaction [1], which represents both pulse amplitude modulation (PAM) and pulse width modulation (PWM) [2].

In recent times, different aspects of light from the easily available Indian species of firefly *Luciola praeusta* have been investigated, and interesting conclusions drawn from these investigations [1-10]. Very recently, another Indian species of firefly *Asymmetricata circumdata* (Motschulsky) has been found, and characteristics of light from male specimens of this species documented [11]. This species, very small in number, dwells in jungles and it is easier to catch the males which come flying out of it just after the sunset. Females stay inside, cannot fly like the males, and hence it is extremely difficult to catch them. Anyway, a survey of literature on firefly bioluminescence indicates that no detailed studies on emissions from female specimens have been carried out till now. This could be due to the fact that flying specimens, which are males, catch our eyes and attract the attention naturally. In this work, an attempt has been made to compare bioluminescence emissions of female specimens of the firefly-species *Asymmetrica circumdata* with those of the male ones.

2 Experimental procedure

Female specimens were caught inside the jungle at Khatkhati, near the Garo Hills in Meghalaya, about 60 km southwest of Gauhati University, India. One such specimen is shown in Fig 1. Due to the extremely small number as well as the high degree of difficulty and risk encountered inside the jungle, a total of twelve

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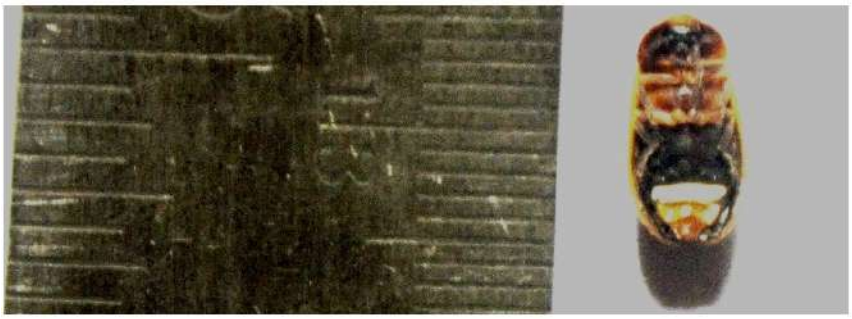
female specimens only could be collected over two summers. They remained alive in captivity for about a day only, and light emission stopped by the time they were brought to the laboratory at Gauhati University from their habitat. Hence the experiments were carried out in a nearby house from 19.00 to 22.00 hours, at temperatures ranging from 28 to 31 °C. A high-resolution spectrometer (Ocean Optics HR4000 series), calibrated earlier with standard lines produced from an iron arc, was used for recording emission spectra. A flashing specimen was kept immobile using sponge and sellotape with its lantern positioned towards the entrance face of the fiber optic cable. Because of the low intensity of the emitted light, the integration time of the spectrometer was set at 2000 ms.

(a)



(b)

Fig 1. A female specimen of the Indian species of firefly *Asymmetricata circumdata* (Motsculsky). (a) Dorsal side, (b) Ventral side.

In the case of recording of flashes of the firefly, a flashing specimen was fixed in a thick piece of sponge using sellotape with its lantern positioned in front of a photomultiplier tube (Hamamatsu H10722 with power supply C10709). 0.22 to 0.35 V control voltage was applied in the photomultiplier tube. The pulses were observed using a digital storage oscilloscope (Tektronix TDS 2022C), and were saved with .CSV file extensions in an external devise HP USB. A digital thermometer was made by using IC LM35 connected to a multimeter (MASTECH MAS 83L), and placed adjacent to the fixed firefly for noting down the temperature. The resolution of this thermosensor was 0.5 °C.

In the case of recording of the diffraction pattern of the light of the firefly, an analytical grating (Hilger) of 15,000 lines/inch was used. Distance of the grating from the light-emitting organ of the firefly was approximately 2.5 cm. A Nikon D7000 camera fitted with AF-S NIKKOR 50 mm 1:1.4 G lens was used to photograph the diffracted light. Distance of the lens of the camera from the grating was approximately 10 cm.

3 Results and discussion

It is worth mentioning that in the case of females only one segment in the lower abdomen emits light, while for males two segments constitute the lantern. An emission spectrum of a female specimen of the firefly-species is presented in Fig 2. The profile is similar in characteristic to that of the males of this

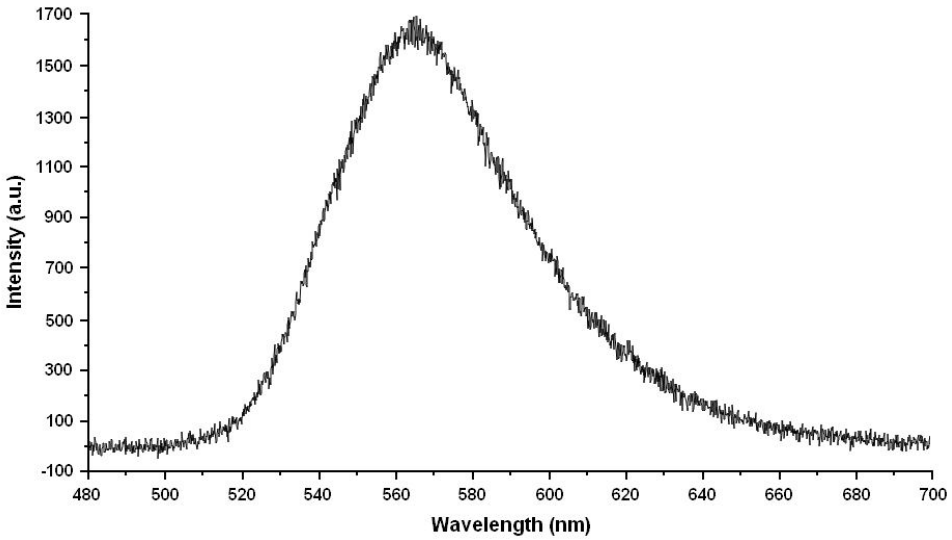


Fig 2. Emission spectrum of a female firefly of the species *A. circumdata* (Motsch.).

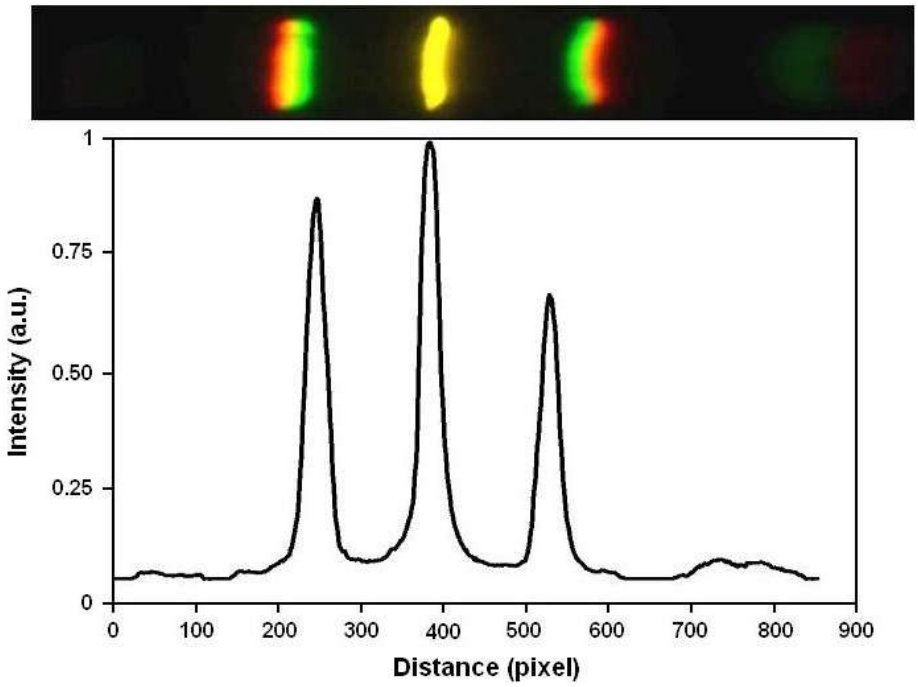


Fig 3. Diffraction pattern produced in a grating (above) with its intensity profile (below).

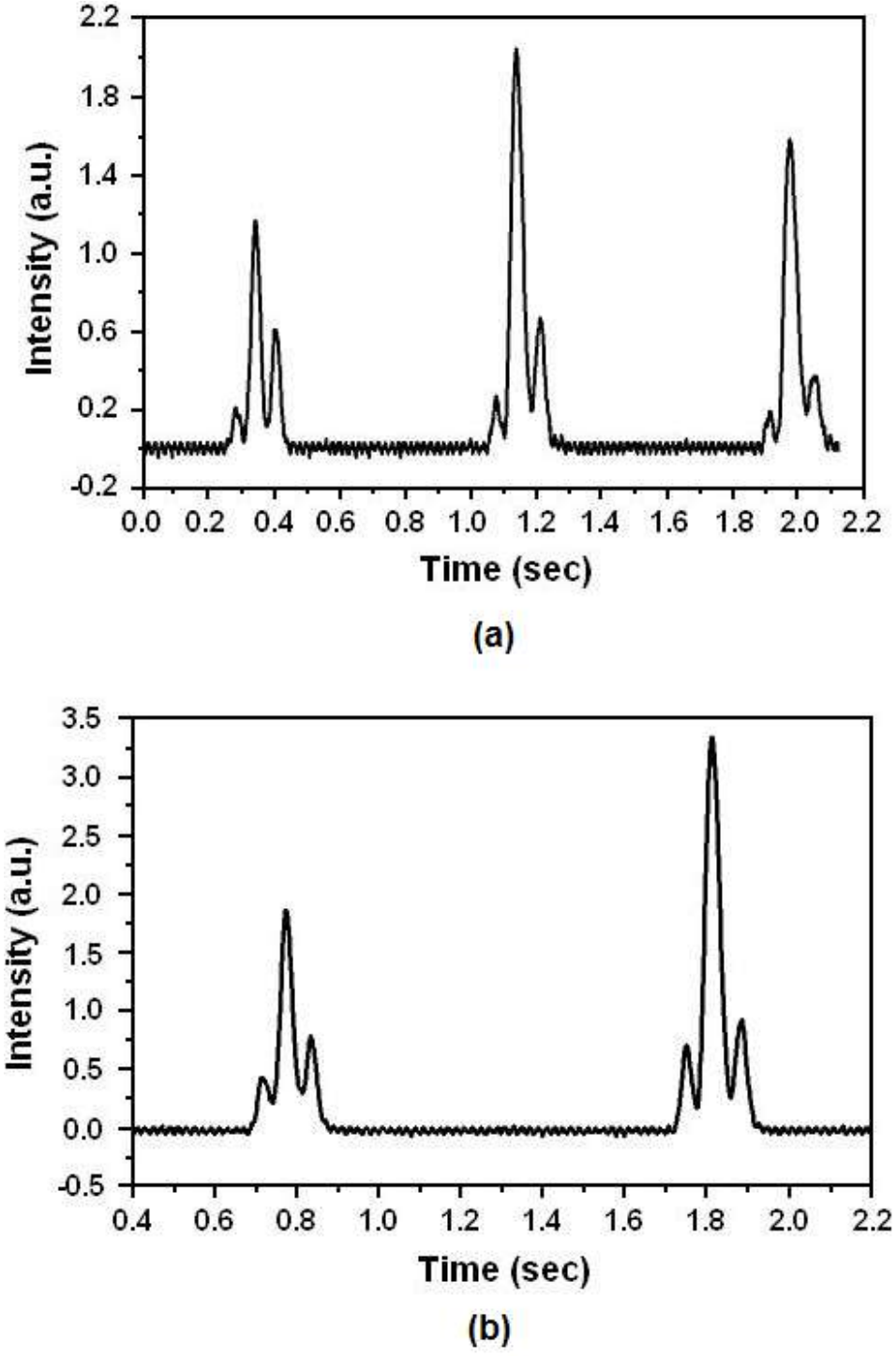


Fig 4. Flashes from two different females of the species.

species [11]. The full width at half maximum (FWHM), spreading from 545 nm to 602 nm, comes out as 57 nm, equal to that of the males. There is one difference, though: the peak appears at 565 nm in contrast to that for the males appearing at 570 nm. There are more than 2000 species of fireflies throughout the world, and they emit light in slightly different wavelengths because of slight differences in their enzyme structures [12]. Hence we may infer that the enzyme structures of males and females of this species differ slightly.

A diffraction pattern recorded in the grating is presented in Fig 3. As is evident, there is only one color-sector in the central principal diffraction maximum which is yellow. In the first order maximum, green and red color-sectors manifest and the yellow sector gets narrowed down. In the barely-visible second order, the yellow one is hardly seen as the other two sectors become considerably broader. As no lens is used in the experiment, the spectrum is an impure one. In this diffraction pattern, three colors, deviated by different amounts, overlap, and so the dominant color at a particular location is the one with the greatest intensity. Hence it gives a good representation of the relative intensity spreads in the wavelength sectors — in particular, that of the most intense yellow portion. As a matter of fact, it has already been inferred that firefly light has a tendency for spectral narrowing in the narrow yellow sector [4].

Flashes from two specimens are presented in Fig 4(a) and (b). In contrast to the male flashes revealing a bi-modal feature [11], the female flashes are characterized by a tri-modal feature. In a 'combination' flash, the central pulse has the maximum intensity by far. The first pulse is considerably weaker than this one, and the third one is observed to be slightly stronger than the first one. All three pulses are triangular in shape. We may hypothesize that there are basically three groups of luminescent luciferin molecules — the second group has the maximum number, of course — which emit at slightly different times. Depending on the time delay of the second and third pulses, the flash duration varies widely. Because of the widely varying times of emission, measurement of the interpulse separation could also be considered as pointless.

In conclusion, female fireflies of the species *A. circumdata* are found to emit at a lower peak wavelength than the males. Their flashes also reveal a different characteristic, namely, tri-modal pattern. These necessitate investigation on their enzyme structure vis-à-vis that of males. Comparative studies on male and female emissions of other species of fireflies are needed to be performed to arrive at a general conclusion.

Acknowledgement

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References

1. Gohain Barua A, Rajbongshi S, The light of the firefly under the influence of ethyl acetate, *J Biosci*, 35(2010)183-186.
2. Gohain Barua A, Modulations in the light of the firefly, *J Biosci*, 38(2013)9-12.
3. Gohain Barua A, Hazarika S, Saikia N M, Baruah G D, Bioluminescence emissions of the firefly *Luciola praeusta* Kiessenwetter 1874 (Coleoptera: Lampyridae: Luciolinae), *J Biosci*, 34(2009)287-292.
4. Dehingia N, Baruah D, Siam C, Gohain Barua A, Baruah G D, Purkinje effect and bioluminescence of fireflies, *Curr Sci,* 99(2010)1425-1427.
5. Gohain Barua A, Iwasaka M, Miyashita Y, Kurita S, Owada N, Firefly flashing under strong static magnetic fields, *Photochem Photobiol Sci*, 11(2012)345-350.
6. Gohain Barua A, Sharma U, Phukan M, Hazarika S, Sharp intense line in the bioluminescence emission of the firefly, *J Biol Phys*, 40(2014)267-274.
7. Sharma U, Phukan M, Rabha M M, Gohain Barua A, Diffraction of the light of the firefly by a grating, *Asian J Phys*, 23(2014)833-837.

8. Sharma U, Goswami A, Phukan M, Rajbongshi S, Gohain Barua, A, Temperature dependence of the flash duration of the firefly *Luciola praeusta*, *Photochem Photobiol Sci,* 13(2014)1788-1792.
9. Muthukumaran T, KrishnaMurthy N V, Sivaprasad N, Sudhaharan T, Isolation and characterization of luciferase from Indian firefly, *Luciola praeusta*, *Lumin*, 29(2014)20-28.
10. Sharma U, Goswami A, Rabha M M, Gohain Barua A, In vivo bioluminescence emissions of the firefly *Luciola praeusta* at low temperatures, *J Photochem Photobiol, B: Biology*, 161(2016)383-386.
11. Goswami A, Sharma U, Rabha M M, Rajbongshi S C, Gohain Barua A, Steady-state and time-resolved bioluminescence of the firefly *Asymmetricata circumdata* (Motschulsky), *Curr Sci*, 109(2015)1838-1842.
12. Seliger H H, Buck J B, Fastie W G, McElroy W D, The spectral distribution of firefly light, *J Gen Physiol*, 48(1964)95-104.

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