

Circular polarization observed in bioluminescence

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While investigating circular polarization in luminescence^{1,2}, and having found it in chemiluminescence^{3,4}, we have studied bioluminescence because it is such a widespread and dramatic natural phenomenon^{5,6}. We report here that left and right lanterns of live larvae of the fireflies, *Photuris lucifera* and *Photuris versicolor*, emit circularly polarized light of opposite sense.

Firefly larvae (Fig. 1) were gathered in the US and sent by airmail to The Netherlands. They were alive up to and during most of the experiments. In our first attempts to detect polarization of luminescence, we measured the total light emission from both lanterns of the larvae. The results were puzzling and disappointing; vanishingly small circular polarization was observed, with only a few exceptions. To our surprise, we noted-fortuitously-that measurements of the light emission from the left or right lanterns separately gave more constant and encouraging results. During the latter experiments one important fact was established. By shifting and rotating the lanterns through many angles and positions with respect to the optical axis of the apparatus, we found that orientational artefacts could not be the origin of the circular polarization subsequently measured. Whereas in these early experiments we used excised lanterns rigidly mounted in a cell, in the later experiments we preferred to attach entire larvae with fine wire to a holder. The holder was positioned in front of a diaphragm so that one lantern only was fixed onto the optical axis of the apparatus. Continuous bioluminescence, lasting from several minutes to an hour, was achieved by feeding the larvae with a concentrated solution of racemic amphetamine hydrochloride in water. Luminescence usually began 5-30 min after the addition of amphetamine. Measurements were then made immediately.

The circular polarization of luminescence was measured as the anisotropy factor or g_{lum} factor $(I_L - I_R)/\frac{1}{2}(I_L + I_R)$. The difference in the numbers of left and right circularly polarized

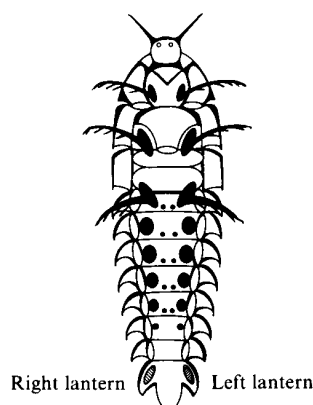


Fig. 1 Sketch of the firefly larva (ventral view). The shaded spots at the bottom represent the lanterns.

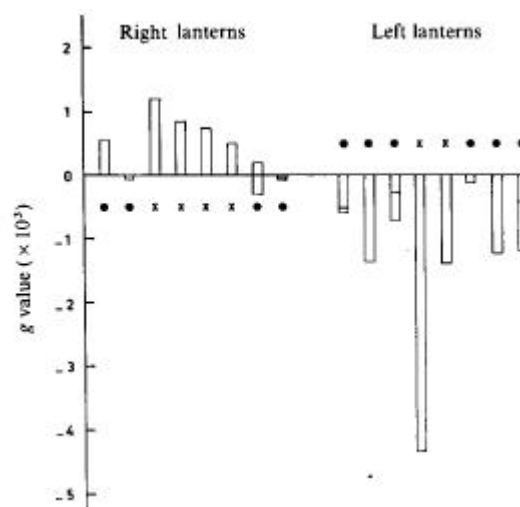


Fig. 2 Results of measurements of circular polarization of bioluminescence of firefly larvae lanterns with sufficiently high emission intensity. The height of each bar represents the g value for an individual lantern of *Photuris lucifera* (X) or *Photuris versicolor* (•). Superimposed bars refer to the effect of orientational changes (see text). The circular polarization is measured at the peak of the emission band at a wavelength of 540 nm using a spectral bandwidth of 40 nm. The r.m.s. noise level corresponds with $g \leq 5 \times 10^{-4}$.

photons emitted, $I_L - I_R$, was modulated at a frequency of 50 kHz and detected as alternating photocurrent; the average luminescence $\frac{1}{2}(I_L + I_R)$ was measured as direct photocurrent^{1,2,7-9}. Measurements of circular polarization of bioluminescence (CPBL) of 16 firefly larvae lanterns are shown in Fig. 2, revealing that the left and right lanterns emit polarized light of opposite sense. Within each group there is a considerable variation in the total light emission and values of g for the individual lanterns. In these experiments, the construction of the sample holder prevented a detailed investigation of orientational effects. However, on the basis of results of earlier experiments, we believe that neither position effects nor those due to linear polarization could have decisively affected our result.

What is the origin of the CPBL, and what is the origin of the difference in handedness shown by the left and right lanterns? According to the accepted mechanism of firefly bioluminescence mediated by luciferin, an achiral emitting state, III* (see Fig. 3), is involved^{5,10-13}. This achiral excited state, by itself, offers no possibility of explaining the CPBL. It may be argued however, that the true excited state consists of a complex between the achiral III* and the enzyme luciferase. Such a chiral excited state complex should, in principle, be capable of emitting circularly polarized light^{3,4}. A second explanation would invoke a circularly dichroic medium capable of partially absorbing the emitted light. Neither of these two modes can explain why the left and right lanterns emit polarized light of opposite sense, unless enantiomeric enzymes, active sites or membranes are proposed. In the absence of any evidence for enantiomeric structures, we are forced to conclude that the CPBL of opposite sense must, at least in part, be based on a macroscopic phenomenon rather than on molecular chirality.

We propose the following hypothesis. The light from a bioluminescent emitter may be (partially) plane polarized due to anisotropy of an absorbing medium or, more likely, by inhomogeneous formation of excited states due to local molecular organization within a photocycle¹⁴. This plane polarized light will become elliptically polarized when it passes through a linearly birefringent medium. Orientated biopolymers can serve as such a medium. On a macroscopic scale the larvae, like many living organisms, have a symmetry plane dividing the lanterns. In this

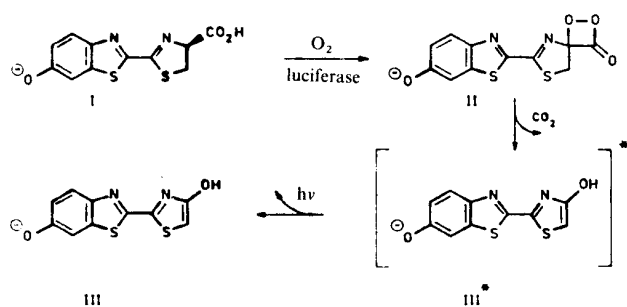


Fig. 3 Schematic mechanism of luciferin/luciferase bioluminescence. Oxygenation of luciferin (I) yields the intermediate II which in turn releases CO_2 , resulting in the production of III (or, by H^+ -abstraction, the enol dianion) in an electronically excited state. This state emits a quantum of light as it goes to its electronic ground state.

sense the lanterns are enantiomeric, even though their constituent molecules are of the same chirality; they may be viewed as macroscopic meso-structures. It is reasonable to assume that this macroscopic mirror image relationship holds on the level of the membrane structure and orientation of the emitters. This will result in circularly polarized light of opposite sense. The emitters from one lantern emit partially plane polarized light making angle $+X^\circ$ with the fast axis of its linear birefringent

medium. The result is ellipticity of one sense ($+Y$). The mirror image relationship of the emitters in the other lantern results in partially plane polarized light with angle $-X^\circ$ with respect to the fast axis of its linear birefringent medium. This must result in ellipticity of opposite sense ($-Y$). We may expect, then, that the meso-orientated lanterns in any bioluminescent organism will emit circularly polarized light of opposite handedness, provided the chirality on the molecular level does not exceed the effect. The results described above suggest experiments dealing with the linear polarization of firefly larvae bioluminescence. Such experiments are planned.

Received 29 February; accepted 13 June 1980.

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