J. exp. Biol. (1979), 79, 225–233
With 9 figures
Printed in Great Britain

PHOTOBIOLOGY OF THE CHLOROPLAST HOSTING MOLLUSC *ELYSIA TIMIDA* (OPISTHOBRANCHIA)

BY M. RAHAT AND EDNA BEN-IZHAK MONSELISE
Department of Zoology, Hebrew University of Jerusalem, Israel

(*Received* 27 *June* 1978)

SUMMARY

Photoreactive behaviour of *Elysia timida* was examined with regard to the posture of its parapodia, covering or exposing its symbiotic chloroplasts. In the dark, the parapodia are held dorsally close together, but they open and spread out in light, at 3×10^3 to 3×10^5 ergs.cm^{-2}.s^{-1}. At higher light intensities (up to 6×10^5 ergs.cm^{-2}.s^{-1}) the parapodia close up again. This behaviour is identical in normal and eyeless individuals. At a wavelength of 540 nm, the slugs open their parapodia as above, but the eyeless *E. timida* will not close their parapodia at the higher light intensities at this wavelength. At 445 and 650 nm, no photoreaction is observed.

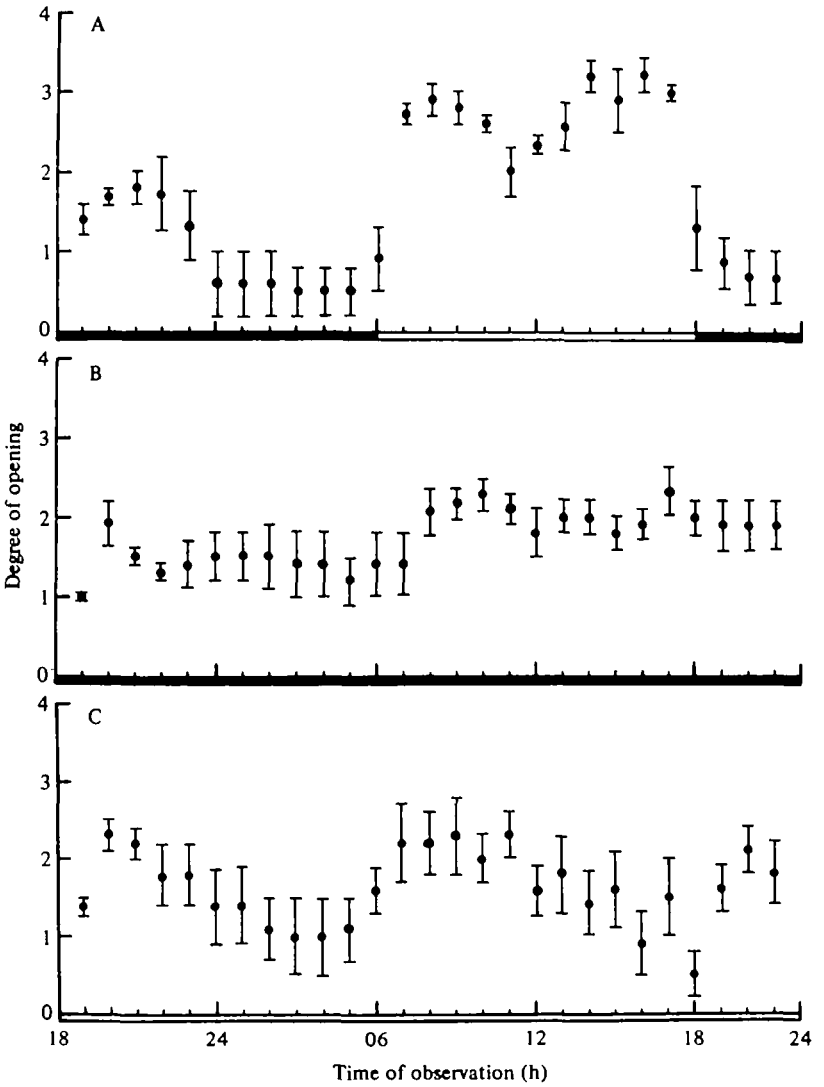
It is postulated that *E. timida* has three photoreceptor systems. (1) An extraocular photoreceptor that differentiates between light and dark, which causes, respectively, opening or closing of parapodia. (2) The eyes, which can differentiate between optimal and above optimal light intensities (the latter causing closure of the parapodia). These photoreceptors perceive light at 540 nm, and could thus contain carotenoid photoreactive pigments. (3) An extraocular photoreceptor, which, like the eyes, can differentiate above optimal light intensities and cause parapodial closure, but only in 'white' light. An interaction of the first two photoreceptors could be an alternative for the function of the latter.

The form or location of the extraocular photoreceptors is unknown. It is proposed that although the symbiosis of algal chloroplasts and *E. timida* might be of recent evolution, this symbiosis has already affected the behaviour of the slug.

INTRODUCTION

Much interest has been centred recently on the naked elysioid slugs that host functional symbiotic chloroplasts in cells of their digestive tract. Trench (1975), has reviewed the occurrence, morphology and biochemistry of this symbiosis. No study however has been made of the effects of this extraordinary plant organelle-invertebrate symbiosis on the behaviour of the host.

Elysia timida (Risso, 1818) is found on rocks and algae along the Mediterranean coast of Israel. A recent publication described its symbiotic chloroplasts and life cycle (Rahat, 1976). Our observations show that this slug changes the position of its parapodia between a contracted, or arrow-like form, and a fully spread posture resembling a flattened leaf (Fig. 1). The aim of this study was to identify the factors that regulate this behaviour.



Figs. 2–4. Extent of parapodial opening under different light (L) and dark (D) conditions. Abscissa: □, L at $2{\cdot}5 \times 10^3$ ergs.cm^{-2}.sec^{-1}; ■, D. Ordinate: Degree of parapodial opening.

Fig. 2. Effect of D–L cycle (*A*) and continuous D (*B*) or L (*C*). Each point represents the mean ± S.E. of 27 *Elysia*.

METHODS

Elysia timida was collected and used within 24 h, unless otherwise stated. In the laboratory, specimens were kept, at 20 ± 2 °C, in covered 100 × 60 mm crystallizing dishes, containing sea water and fronds of various algae under continuous white fluorescent light (at $2{\cdot}5 \times 10^3$ ergs.cm^{-2}.s^{-1}), or as required for experimental purposes. Light intensity was measured with a YSI-Kettering model 65 Radiometer. For experiments requiring different light intensities, a 6 V car lamp was used with variable transformer. High intensities were obtained by passing light through a 100 mm

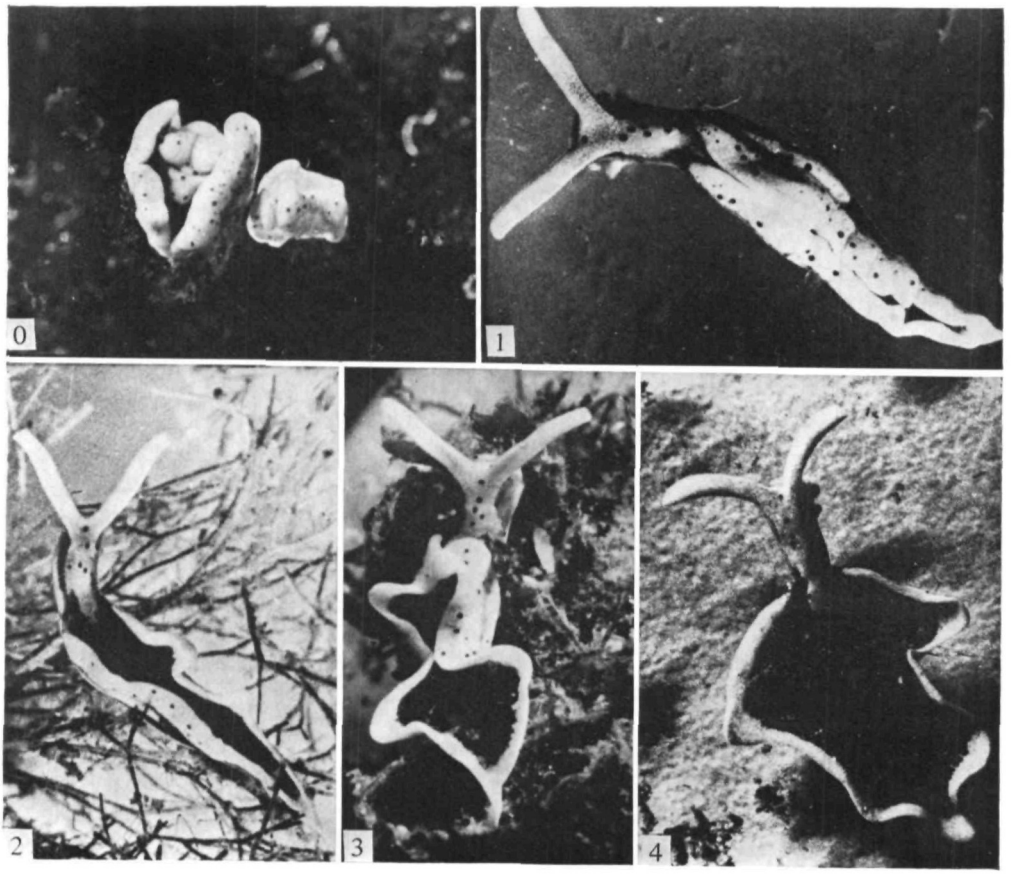


Fig. 1. *Elysia timida*: forms of parapodial position. Numbers 0–4 were used to designate degrees of relative parapodial opening.

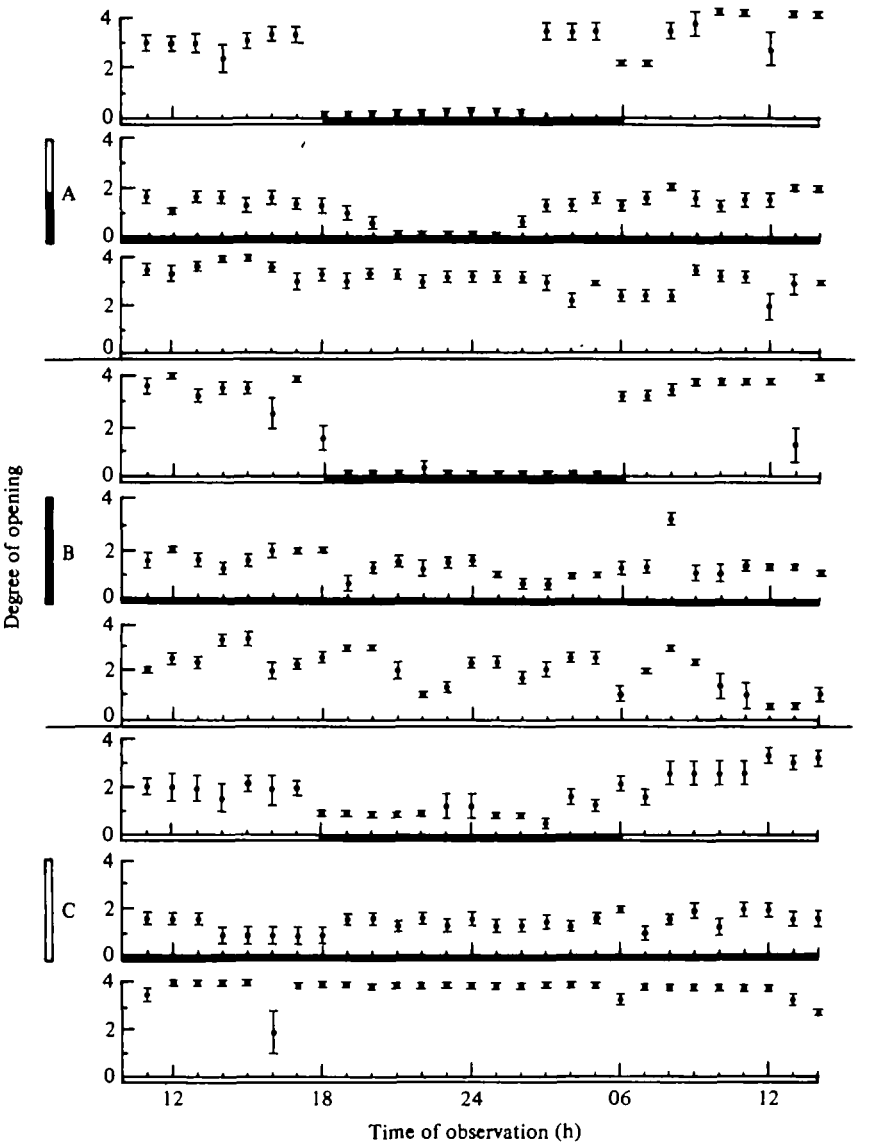


Fig. 3. Effect of L–D preincubation. Three groups of nine *Elysia* were kept for 6 days respectively in a 12–12 h L–D regime (*A*), in continuous D (*B*), or in L (*C*). Each group was then divided into three subgroups that were subjected to one of the above treatments respectively, for 28 h. Each point represents the mean ± S.E. of three *Elysia*.

magnifier and a heat filter. Interference type Balzer broad band K-type filters were used for defined wavelength irradiation (Balzer, Lichtenstein). Readings in the 'dark' were made in dim light, below the readability of the Radiometer.

To remove the eyes the animals were relaxed in sea water containing 0·05% Menthol (Merck) and 0·1% MS-222 (Sandoz, ethyl-*m*-aminobenzoate). A transverse incision was made on the dorsal surface, between the eyes, which were then pulled out by their stalks using a watchmaker's forceps. In control animals, a similar incision

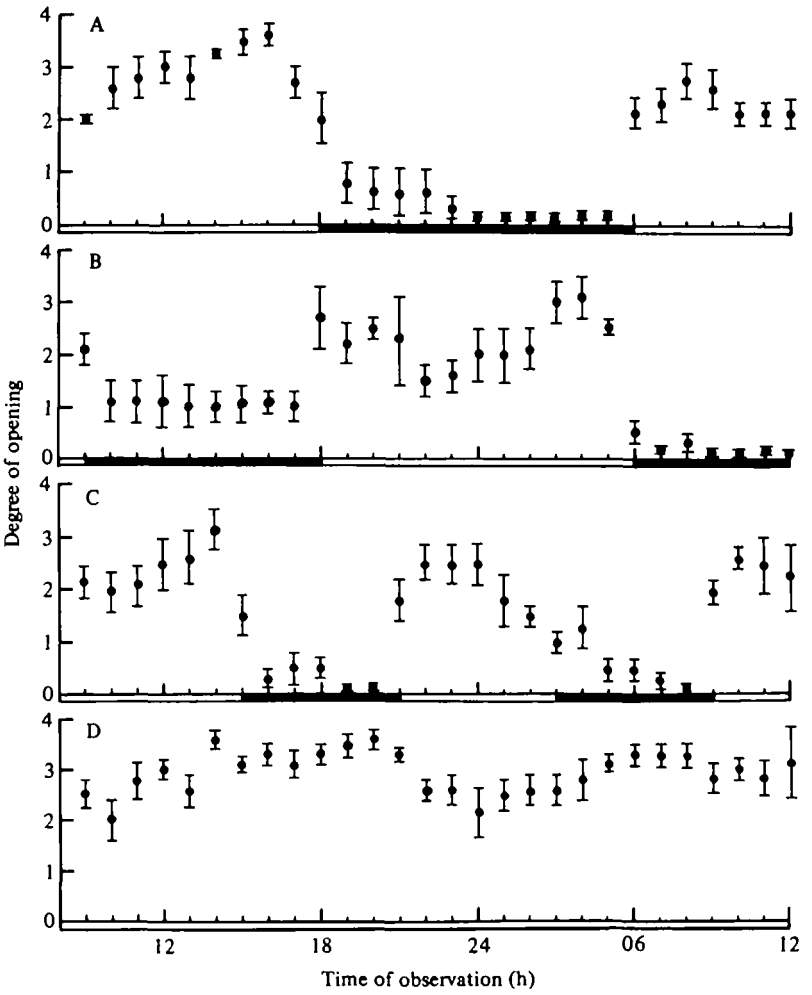


Fig. 4. L–D periods were shifted from normal day–night time, as shown on the abscissa. Each point represents the mean ± S.E. of seven *Elysia*.

was made but the eyes were left untouched. After 24 h, no scar could be seen where the cuts had been made (Fig. 6).

RESULTS

Response to light and darkness

Parapodia were observed to close in the dark (D), and open up in light (L) (Fig. 2*A*). In continuous darkness or light, however (Fig. 2*B* and *C*), some fluctuations occurred, the parapodia opening more during real-day time (06·00–18·00 h) than during the night (18.00–06.00 h). This could result from the involvement of a biological clock which influences parapodial behaviour.

To test this hypothesis, *Elysia timida* were pre-incubated for 6 days in different L–D regimes (Fig. 3), and were then subjected to varied L–D regimes for 28 h, while their parapodial positions were recorded. An unequivocal parapodial response to a

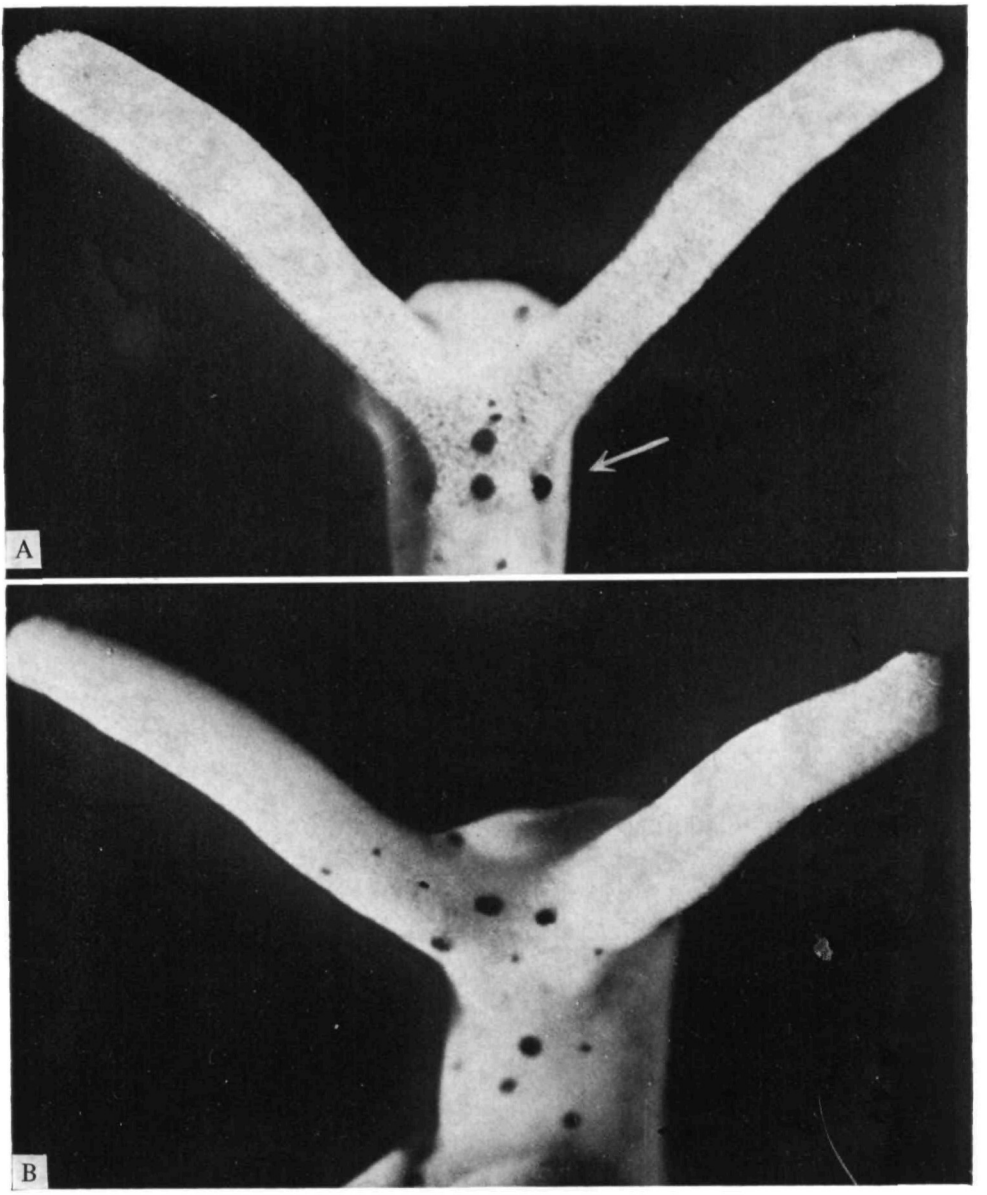


Fig. 6. Normal (*A*) and eyeless (*B*) heads of *Elysia*. Arrow in (*A*) points to eye. Yellow, red and black pigment spots, appear in photo as dark dots.

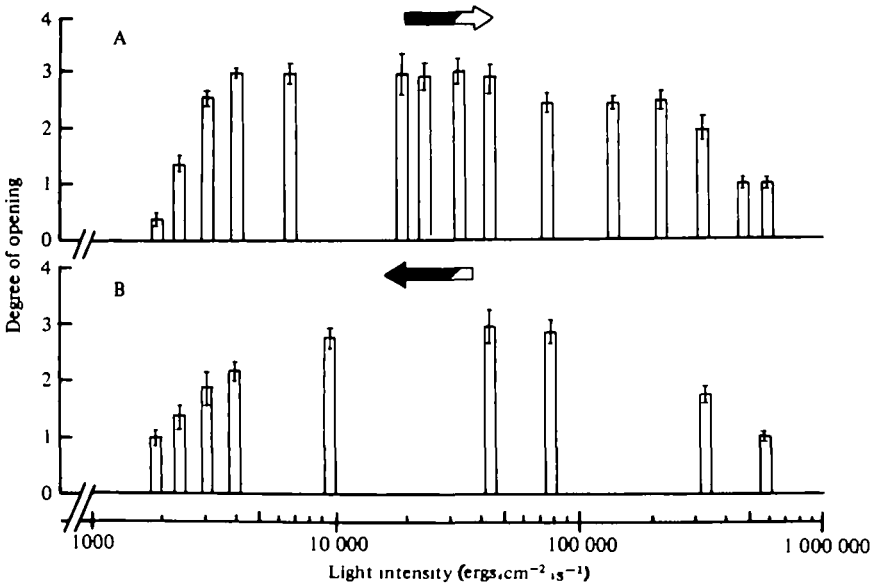


Fig. 5. Effect of increasing L (*A*), followed by decreasing illumination (*B*). Light intensities were changed every 10 min, and the degree of parapodial opening was recorded at the end of this period. Each point represents the mean ± S.E. of 27 *Elysia*, observed in five separate experiments. The slugs were kept in the laboratory for 2–6 weeks prior to these experiments.

L–D change could be seen in all *Elysia*, which was independent of the pre-incubation regime (Fig. 3*A*, *B* and *C*, upper line). A parallel, although smaller, change in parapodial position (Fig. 3*A*, middle line), was, however, also observed if the slugs were placed in continuous D, after 6 days in a 12–12 h L–D regime. Fluctuations in parapodial opening were also seen if *Elysia* were pre-incubated in continuous D and then placed either in D or in continuous L (middle and lower line in *B*). After pre-incubation in continuous L (*C* in Fig. 3), an unchanging response to either L or D was observed. Parapodia were relatively closed in D and open in L (middle and lower line in *C*).

The effect of a possible circadian cycle on parapodial position in *Elysia* was further examined by reversing 'day' and 'night', in relation to the natural cycle (*A* and *B*, Fig. 4), or by imposing 6–6 h L–D periods (*C* in Fig. 4). A control (D) was retained in continuous light. Under these regimes *Elysia* opened their parapodia in L and closed them in D, independently of the time or duration of the illumination period.

Effect of different light intensities

E. timida were exposed to increasing and decreasing light intensities (Fig. 5). Parapodia remained closed in dim light, up to about 3×10^3 ergs.cm^{-2}.s^{-1}, opened at higher light intensities, and closed again at above 3×10^5 ergs.cm^{-2}.s^{-1}. This behaviour was identical whether light intensity was increasing (*A*) or decreasing (*B*).

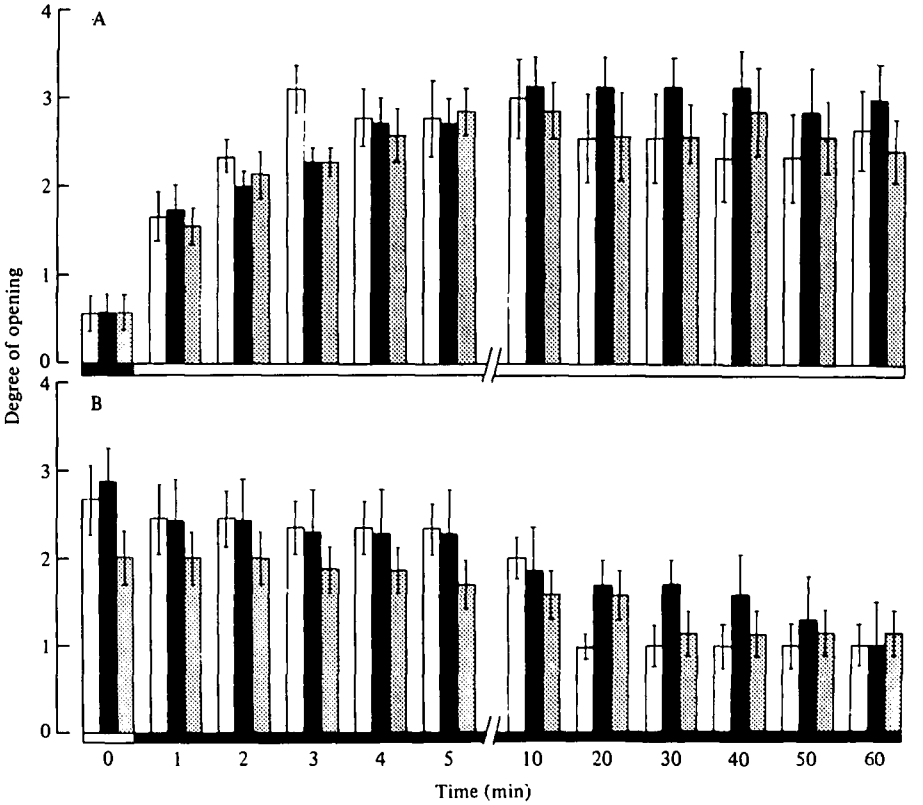


Fig. 7. Reaction time to D–L (*A*) and L–D (*B*) changes, in normal □, eyeless ■, and sham-operated ▦ *Elysia*. Each column represents the mean ± S.E. of nine *Elysia*. L = $2{\cdot}5 \times 10^3$ ergs.cm^{-2}.s^{-1}.

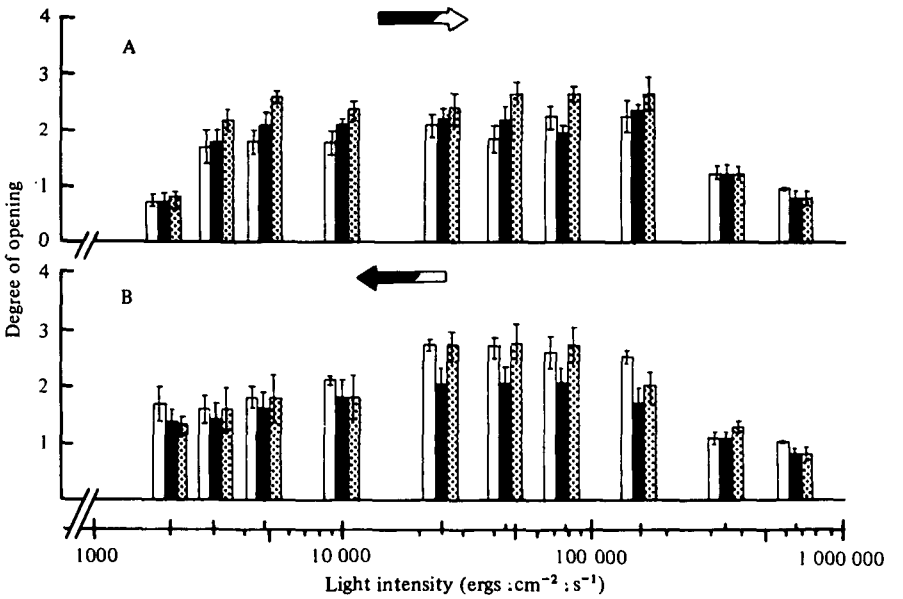


Fig. 8. As in Fig. 5. Each column represents the mean ± S.E. of ten normal □, eyeless ■, or sham-operated ⊡ *Elysia*.

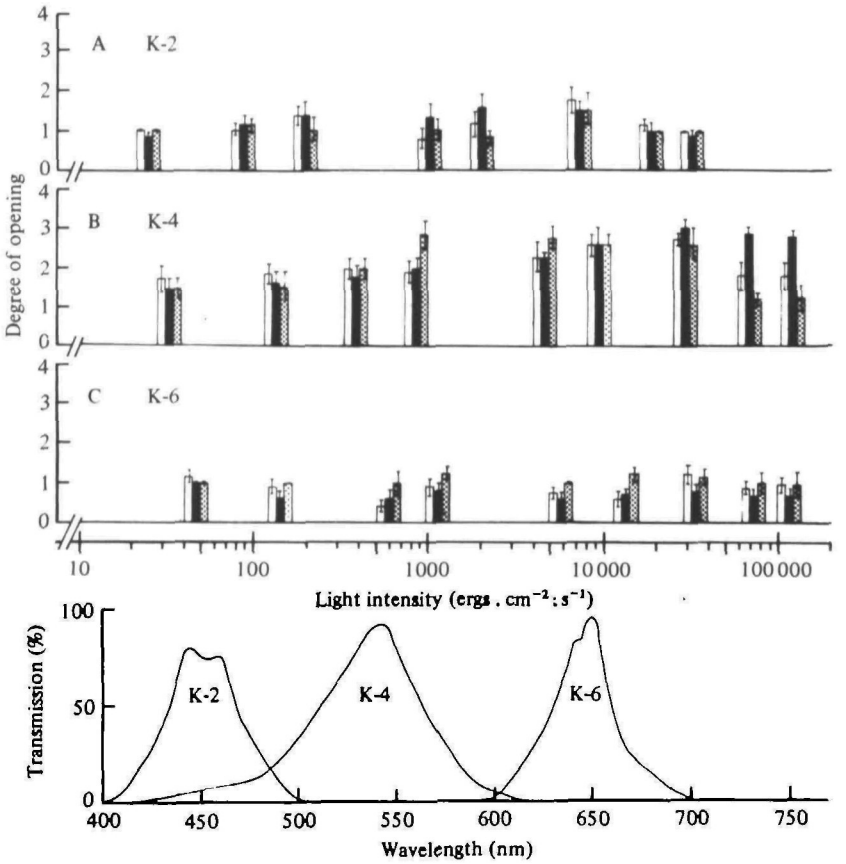


Fig. 9. Effect of increasing L intensities, at different wavelengths, on parapodial opening (upper part of Figure). Each column represents the mean ± S.E. of eight normal □, eyeless ■, or sham-operated ⊡ *Elysia*. Lower part of Figure: relative transmission of filters used in this experiment (Balzer broad band K-type, Lichtenstein).

Role of eyes as photoreceptors

Three groups of ten *Elysia* were used, of which one group was left as a control, one was sham-operated, and the third had their eyes removed as described in Methods (Fig. 6). The three groups were then exposed to D–L and L–D changes (Fig. 7). Reaction time and form were about the same in all three groups. On transfer from D to L, the parapodia opened within 5 min. On transfer from L to D, it took the normal *Elysia* about 30 min to attain a closed position. The eyeless *Elysia* closed their parapodia fully after only 60 min. The three groups of *Elysia* were then exposed to increasing and decreasing light intensities, as described above. All three groups behaved in a similar manner: parapodia opened at about 3×10^3 ergs.cm^{-2}.s^{-1}, and closed again at light intensities above 3×10^5 ergs.cm^{-2}.s^{-1} (Fig. 8).

Identification of additional photoreceptors

The above experiment was repeated (with the three groups of *Elysia*) at three different wavelengths, using Balzer colour filters with maximal transmission at 445,

540 and 650 nm respectively (Fig. 9). At around 445 and 650 nm, the slugs did not react to light, even at the highest intensities. At around 540 nm, however, all *Elysia* opened their parapodia at about 3×10^3 ergs.cm^{-2}.s^{-1}, as they did in 'white' light and those with eyes closed their parapodia again at above 5×10^4 ergs.cm^{-2}.s^{-1}. The eyeless *Elysia* did not close their parapodia, even above 10^5 ergs.cm^{-2}.s^{-1}.

DISCUSSION

Elysia timida shows different postures of its parapodia, under different light conditions. The parapodia spread out at optimal light intensities of about 3×10^3 to 10^5 ergs.cm^{-2}.s^{-1}, and close at lower or higher light intensities (Figs. 1–5). Parapodial position of *E. timida* can thus be taken as an indicator of light perception in this slug.

What are the receptors and regulators of such behaviour? From studies on plant behaviour, it is known that plants change the position of their leaves, and even the position of chloroplasts within their cells, in response to changes in light direction and intensity (Mayer, 1971; Galston & Satter, 1976). An analogous conclusion might be drawn for the chloroplast-hosting *E. timida*. The symbiotic chloroplasts are obviously protected from mechanical or irradiation damage when the parapodia are closed, respectively, in the dark or in excessive light (Fig. 5). At optimal light intensities, however, the chloroplasts are exposed to enable them to photosynthesise.

Our results indicate that there might be some circadian effect on parapodial position during the day or night (Figs. 2 and 3). However, from results shown in Fig. 4, it must be concluded that the direct effect of light overrules any other influence although the presence of a circadian clock cannot be ruled out.

The rate and form of photoreactive behaviour, was the same for all *Elysia* tested, whether normal or eyeless (Fig. 6). Reaction times to D–L or L–D changes (Fig. 7), and parapodial position in D or up to the highest light intensities (Fig. 8), were, also, not affected by removal of the eyes. It must, therefore, be concluded that for the opening and closing of parapodia, at different 'white' light intensities, eyes are not required. The presence of other photoreceptors must therefore be assumed.

In *E. timida*, the most obvious additional photoreceptors are the symbiotic chloroplasts. The excitation of the chlorophyll by light, could possibly be transmitted to the slug, which would then react by regulating its parapodial position. However, no reaction to light was observed, either in the normal or in the eyeless slugs, at 445 and 650 nm (the wavelengths absorbed by chlorophylls) even at 10^6 ergs.cm^{-2}.s^{-1} (Fig. 9*A* and *C*). It must thus be concluded that the excitation of chlorophylls by light cannot be transmitted to the slug.

In the region of 540 nm (the wavelength absorbed by carotenoid pigments), as in 'white' light, the slugs reacted to the light, and opened their parapodia at increasing intensities, but only those with eyes closed again at the highest light intensities (Fig. 9*B*). It is, therefore, the eyes that perceive the changes at high light intensity at this wavelength. This perception is, apparently, conducted to some effector that causes closure of the parapodia.

We thus propose that in *E. timida* there are three photoreactive systems. One, an extraocular qualitative photoreceptor, perceives the difference between light and dark,

and causes respectively the opening and closure of the parapodia. The second, the eyes, can differentiate between optimal and higher light intensities, the latter causing closure of the parapodia. These photoreceptors perceive light at 540 nm, and could be based on a carotenoid photoreactive pigment. In plants, flavins having a maximal absorption at 450 nm, have been suggested as photoreceptors which induce chloroplast movement (Mayer, 1971). The third proposed photoreceptor, like the first, is extraocular. Like the eyes, it can also perceive above-optimal light intensities and causes closure of the parapodia. This photoreceptor differs however from the eyes, as it only reacts to 'white' light. An alternative to the presence of a third photoreceptor, would be to postulate an interaction between the first two photoreceptors, at above optimal light intensities. Further research should clarify these possibilities.

We have yet no data as to the location and form of the extraocular photoreceptors. It has been shown before that eyeless genera of molluscs do react to light (Braun, 1954), and a general dermal light sense has been described for many animals (Steven, 1963). In *E. timida*, an ultrastructural study of its yellow, red and black pigment spots, might perhaps reveal an 'eye spot' structure.

The correlation between 'day' and 'night' light intensities, and parapodial position, could be affected by a biological clock. However, it could be that light directly regulates parapodial position. In both cases, the presence of such a regulatory capacity must be regarded as a preadaptational trait of *E. timida*, as is also the robustness of the chloroplasts, that enables them to survive for 3 months in the cells of their host, after passing through its digestive tract (Schönfeld *et al.* 1973; Trench *et al.* 1973). This trait of *E. timida* can, however, be regarded also as one acquired by mutual co-evolution, after the chloroplast-*Elysia* symbiosis has been established. Regulation of parapodial position by a biological clock would probably indicate a longer evolved symbiosis, while direct photoreaction would suggest a more recent one. The observation that chloroplasts have to be taken up anew, at each generation of *E. timia* (Rahat, 1976), also indicates that this symbiotic association has evolved relatively recently but has, nevertheless, already affected the behaviour of the host.

This study was partially supported by the Israel Commission for Basic Research.

REFERENCES

Braun, R. (1954). Zum lichtsinn der augenlosen Muscheln. *Zool. Jb.* **65**, 194–208.

Galston, A. W. & Satter, R. L. (1976). Light, clocks and ion flux: an analysis of leaf movement. In *Light and Plant Development* (ed. H. Smith), pp. 159–185. London: Butterworth.

Mayer, F. (1971). Light induced chloroplast contraction and movement. In *Structure and Function of Chloroplasts* (ed. M. Gibbs), pp. 35–49. New York: Springer.

Rahat, M. (1976). Direct development and symbiotic chloroplasts in *Elysia timida* (Mollusca, Opisthobranchia). *Israel J. Zool.* **25**, 186–193.

Risso, A. (1818). Mémoire. Sur quelques Gastéropodes nouveaux, Nudibranches et Tectibranches observés dans la Mer de Nice. *J. Physiol., Paris* **87**, 368–377.

Schönfeld, M., Rahat, M. & Neumann, J. (1973). Photosynthetic reactions in the marine alga *Codium vermilara*. I. CO_2 fixation and Hill reaction in isolated chloroplasts. *Plant Physiol.* **52**, 283–287.

Steven, D. M. (1963). The dermal light sense. *Biol. Rev.* **38**, 204–240.

Trench, R. K. (1975). Of 'leaves the crawl': functional chloroplasts in animal cells. *Symp. Soc. Expl Biol.* **29**, 229–265.

Trench, R. K., Boyle, J. E. & Smith, D. C. (1973). The association between chloroplasts of *Codium fragile* and the mollusc *Elysia viridis*. I. Characteristics of isolated *Codium* chloroplasts. *Proc. R. Soc. Lond.* B. **184**, 51–61.