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Photoperiodic receptor in the nymph of *Poecilocoris lewisi* (Heteroptera: Scutelleridae)

RYO MIYAWAKI, SHINICHI I. TANAKA and HIDEHARU NUMATA*

Department of Bio- and Geosciences, Graduate School of Science, Osaka City University, Osaka 558-8585, Japan;
e-mail: numata@sci.osaka-cu.ac.jp

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Abstract. The receptor for photoperiodism in nymphs of *Poecilocoris lewisi* was examined using a phosphorescent paint, which absorbs light energy and emits phosphorescence in the dark. This species shows a facultative diapause in the fifth (final) nymphal instar and its induction is primarily controlled by photoperiod in the fourth instar. The incidence of diapause in the fifth instar was determined after exposing selected regions of the body surface to a longer photophase than the rest by applying a phosphorescent paint in the fourth instar. The incidence of diapause was significantly lower in insects with their compound eyes painted than in control insects at near-critical daylengths. However, painting the central part of the head had no effect. It is concluded, therefore, that the compound eyes are the principal receptor for photoperiodism in nymphs of *P. lewisi*. This is the first report implicating the compound eyes in the reception of photoperiod in nymphal insects.

INTRODUCTION

Receptors of photoperiod have been localized in various insects. In the adult stage, the receptor is the brain or the compound eyes (e. g., Lees, 1964; Numata & Hidaka, 1983; see Numata et al., 1997 for review). In the larvae of Lepidoptera, *in vitro* experiments unequivocally show that photoperiod is perceived by extraretinal photoreceptors in the brain or the brain-suboesophageal ganglion complex (Bowen et al., 1984; Hasegawa & Shimizu, 1987), and the stemmata in *Pieris brassicae* has a subordinate role (Seuge & Veith, 1976). However, the receptors of photoperiod have not been examined in nymphs of Hemimetabola.

In Heteroptera, the compound eyes are the principal receptor of photoperiod in adults of *Riptortus clavatus*, *Graphosoma lineatum*, and *Plautia crossota stali*, although another photoreceptor becomes apparent when the compound eyes are removed in *P. c. stali* (Numata & Hidaka, 1983; Nakamura & Hodkova, 1998; Morita & Numata, 1999). *Poecilocoris lewisi* (Distant) (Heteroptera: Scutelleridae) enters diapause in the fifth (final) nymphal instar induced by a short-day photoperiod (Tanaka et al., 2002). In the present study, the role of the compound eyes in this photoperiodic response was examined by painting the compound eyes of the nymphs of *P. lewisi* with a phosphorescent paint.

MATERIALS AND METHODS

Adults and fifth instar nymphs of *P. lewisi* were collected in the Botanical Gardens of Osaka City University, Katano, Japan (34.8°N, 135.7°E), and their progeny were used for the experiment. Nymphs and adults were reared on peanuts and water (Tanaka et al., 2002), with the addition of 0.05% sodium L-ascorbate and 0.025% L-cystein to the water. The experiment was performed at 25 ± 1°C. White fluorescent lamps covered with white paper were used for the light source and the light intensity in the photophase was 100–150 lx. Nymphs were reared under a long-day photoperiod of 15 h light and 9 h darkness (15L : 9D) until one day after the third ecdysis.

The location of the photoperiodic receptor was determined by applying a phosphorescent paint to selected regions of the body surface, of nymphs kept at various photoperiods. Numata & Hidaka (1983) also used a phosphorescent paint to locate the receptor for photoperiodism in *R. clavatus*. The main component of the pigment they used was ZnS, which absorbs light energy and phosphoresces. Since then, new phosphorescent paints have been developed the afterglow from which lasts much longer and is more intense. The paint used in the present study (N.T. Luminous Paint Type 8H, Toho Co. Ltd., Osaka) contains a pigment of which the main component is $SrAl_2O_4$:Eu,Dy, and the brightness of the afterglow is 10 mcd/m^2 after 1 h.

Fifth instar nymphs were returned to 15L : 9D. The insects that developed into adults within 30 days were regarded as non-diapause nymphs, whereas those that did not were regarded as in diapause, because most nymphs develop into adults about 14 days after the fourth nymphal ecdysis under continuous long-day conditions (Tanaka et al., 2002). Statistical analysis used was the Fisher's exact probability test with a level of significance $p = 0.05$.

RESULTS

Figure 1 shows that *P. lewisi* responded to the photoperiod in the fourth instar, even though they were kept under a long-day photoperiod (15L : 9D) in the other instars. Most nymphs entered diapause in the fifth instar when exposed to 13L : 11D or 14L : 10D in the fourth instar, emerged as adults without entering diapause when exposed to 15L : 9D or 16L : 8D. The critical daylength was about 14.5 h. The incidence of diapause was significantly lower in insects of which the compound eyes were painted with the phosphorescent paint than in intact insects both under 14L : 10D and under 14.5L : 9.5D. The phosphorescent paint on compound eyes shortened the critical daylength by about 0.5 h. In contrast, the incidence of diapause in insects of which the vertex was painted with the phosphorescent paint was not significantly different from that in intact insects, both under 14L : 10D and under 14.5L : 9.5D. There were no significant

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* Corresponding author.

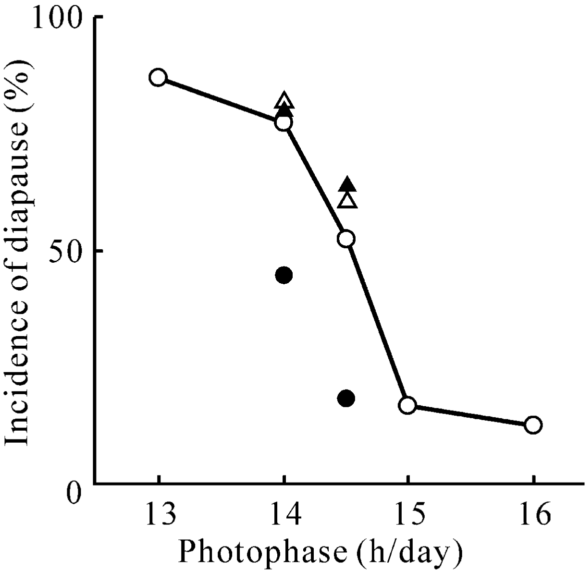

Fig. 1. Effect on the incidence of nymphal diapause in *Poecilocoris lewisi* kept at 25°C of exposing a selected region of the body surface to a longer photophase than the rest by applying a phosphorescent paint. Fourth instar nymphs subjected to various treatments (○, intact; ●, compound eyes painted; △, vertex painted; ▲, compound eyes treated with solvent) were exposed to various photoperiods (n = 18–28). Nymphs were reared under 15L : 9D in the other instars.

differences in the incidence of diapause in intact insects and those of which the compound eyes were treated with only solvent, both under 14L : 10D and under 14.5L : 9.5D. It is concluded, therefore, that the compound eyes are the principal receptors for photoperiodism in nymphs of *P. lewisi.*

DISCUSSION

Cauterization and covering of photoreceptors with an opaque paint have been used to localize the receptor of photoperiod in insects (Numata et al., 1997). However, Geispits (1957) pointed out that light may enter the photoreceptor through the semitransparent cuticle even after it is covered with a black paint. Lees (1964) recognized the defects of elimination experiments and used fine light-guides to focus supplementary illumination on localized sites on the body surface in adults of *Megoura viciae.* Nishiitsutsuji-Uwo & Pittendrigh (1968) reported that paint did not always adhere tightly and occlude light when the compound eyes of *Leucophaea maderae* were painted. Moreover, even if the elimination of a photoreceptor had no effect on photoperiodism, there is still a possibility that two or more receptors are involved and that after elimination of one receptor the other receptor(s) responds to photoperiod (Numata et al., 1997). Exposing a selected region to a longer photophase is a simpler way of resolving this problem (Lees, 1964).

When the compound eyes of adult *R. clavatus* were painted with a phosphorescent paint, the insect responded to the photophase extended by phosphorescence, and it was concluded that the compound eyes are the principal receptor of photoperiod in this species (Numata & Hidaka, 1983). Similarly, it is demonstrated here that the compound eyes perceive photic information responsible for the induction of diapause in nymphs of *P. lewisi.*

Twenty years ago photoreceptors like the compound eyes, stemmata and ocelli were not thought to be involved in the photoperiodic responses of insects (Saunders, 1982). Since then the compound eyes have been shown to have a role in the adults of some insects (Numata & Hidaka, 1983; Shiga & Numata, 1996, 1997; Nakamura & Hodkova, 1998; Morita & Numata, 1999). The present results show that the compound eyes have a role in photoperiodism in *P. lewisi*. This is the first report to locate the receptor of photoperiod in nymphs of Hemimetabola. In larvae of Holometabola the role of the stemmata in the reception of photoperiod has been studied only in Lepidoptera (e. g., Tanaka, 1950; Kono, 1970; Hayes, 1971; Shimizu, 1982). Furthermore, even in *Pieris brassicae*, in which the brain has a predominant role in photoperiodic reception, cauterization of the stemmata changes the spectrum sensitivity for the induction of pupal diapause (Seuge & Veith, 1976). The receptors of photoperiod in larvae or nymphs of Hemimetabola and Holometabola need further study.

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