Threshold and quantitative photoperiodic responses exist in an insect

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Abstract. The photoperiodic response for the induction of adult diapause and that for the determination of nymphal body coloration were compared in *Plautia stali* Scott (Heteroptera: Pentatomidae). The former was an all-or-none response with a distinct threshold, but the latter was a graded response which varied quantitatively with the photophase duration. The photoperiodic clock in this species is discussed.

Key words. Photoperiodic clock; adult diapause; body coloration; threshold response; quantitative response; *Plautia stali.*

Photoperiodic responses control various physiological and morphological characters in insects[1, 2]. Some of the responses result in one of two discontinuous character states, such as wing form, whereas others control characteristics showing continuous variation, such as body size[2]. Responses of the former type usually have one or two distinct thresholds in the photoperiodic response curve. Although some responses of the latter type also have a distinct threshold(s), the others show graded changes with photophase duration[2]. Some authors have examined photoperiodic responses both of a discrete dimorphic character, and of a graded character, in a single species, and reported that both are threshold responses with a common critical photophase(s)[3–5]. Kimura and Masaki[6] reported in *Orgya thyellina* (Lepidoptera: Lymantriidae) that the photoperiodic response curve for pupal melanism (a graded character) resembled that for the determination of diapause or wing form (discrete dimorphic characters) that is controlled by a threshold response, although the degree of melanization varies quantitatively to some extent with the photophase duration around the two critical photophases. However, the quantitative response in this study was not clear, because the pupal coloration was divided into only three grades[6]. We recently showed that in *Riptortus clavatus* (Heteroptera: Alydidae) the photoperiodic response curve for the determination of adult body coloration (a graded character) was similar to that for the determination of adult diapause (a discrete dimorphic character). Both responses were apparently of the threshold type, with common critical photophases. However, within the range close to the critical photophase, the body color responded somewhat quantitatively to the photophase duration[7]. Therefore, it was of interest to compare the photoperiodic responses of similar characters in another species.

The brown-winged green bug (= the oriental stink bug in the USA), *Plautia stali* Scott has a facultative adult diapause which is controlled by photoperiod[8–10]. This is a photoperiodic response of a discrete dimorphic character because there are only two character states, diapause and nondiapause. In this species, however, photoperiod also affects the body coloration of the fifth (final) instar nymphs[11]. This is a response of a graded character because the nymphal body coloration varies continuously between green and dark brown. In this study, we compared these two photoperiodic responses in *P. stali.*

Materials and Methods

Adults of *P. stali* were collected after overwintering on cherry trees in Tawaramoto (34° 30′ N, 35° 45′ E), Nara Prefecture, Japan, in May–June 1987 and 1988, and eggs laid by them were used for experiments. Insects were reared on raw peanuts and water[12] under LD 1:23, 4:20, 10:14, 12:12, 13:11, 14:10, 16:8, or 24:0 at 25 ± 1 °C.

This species lays eggs usually as a mass of 14. Eggs were separated and mixed before the experiments to avoid genetic bias. Because not only photoperiod but also rearing density affects the nymphal body coloration in this species[11], the rearing density was kept constant. Initial density was 14 eggs per 200 ml plastic cup, and the cups in which fewer than 10 adults emerged were discarded.

The period of the fifth nymphal instar was about seven days at 25 °C. Five days after the fourth ecdysis, the nymphs were sexed and their body coloration was classified. The difference in coloration was observed on various parts of the body, not only in melanization of the cuticle but also in the deposition of brown pigment in the epidermal cells, and the nymphs varied from green to a dark brown color. However, because the

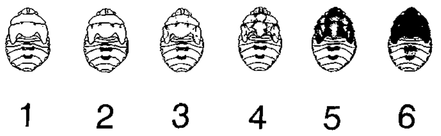

Figure 1. Grades of body coloration in the fifth instar nymphs of *Plautia stali*.

coloration in the abdomen was affected by the stretching of the integument as the nymphs grew, the nymphs were classified into six classes only by the degree of melanization in the pronotal cuticle (fig. 1). Then they were reared as single male and female pairs in 200 ml plastic cups.

These insects were dissected 15 days after adult emergence, and their diapause status was examined. In the females the criterion for nondiapause adults was yolk deposition in the oocytes, whereas in the males the diapause status was judged by the development of the ectodermal accessory gland[10].

Results

Diapause. Long-day conditions (LD 14:10 and 16:8) and continuous light averted diapause, whereas the short-day conditions that naturally occur in autumn (LD 12:12 and 13:11) induced diapause. The critical day-length within the range of natural day-lengths was between 13 and 14 h. From LD 12:12 to LD 1:23, the incidence of diapause gradually decreased as the photophase decreased. There was no notable difference in the diapause incidence between the sexes under the various conditions although several males entered diapause even under LD 14:10 and 16:8 whereas most or all of the females did not (fig. 2).

Body coloration. Because there was no notable difference in the nymphal body coloration between the two sexes, the results for the two sexes were combined. Under typical long-day conditions (LD 16:8) the proportion of light-colored nymphs (grades 1–3) was high, about 80%, whereas under typical short-day conditions (LD 10:14) more than 80% of the nymphs were dark-colored (grades 4–6). Continuous light (LD 24:0) and very short-day conditions (LD 1:23) had similar effects, and gave body colors intermediate between long-day and short-day ones (fig. 3).

The photoperiodic response curves controlling diapause and body coloration were compared within the natural range of photoperiod between LD 10:14 and 16:8 (fig. 4). If *P. stali* uses a common clock system for both responses that only detects whether the photophase is longer than the critical value or not, the photoperiodic response curves for the body coloration should also change sharply between LD 13:11 and 14:10. However, the response gradually changed with the photophase duration: the shorter the photophase, the higher was the proportion of dark-colored nymphs. There was no distinct threshold of photophase.

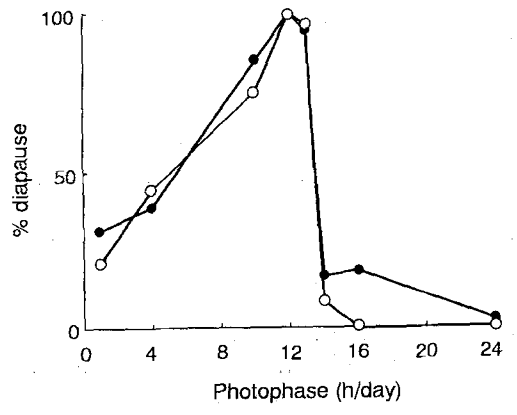

Figure 2. Photoperiodic response curves for the induction of adult diapause at 25 °C in *Plautia stali*. Closed circles, males; open circles, females. n = 27–58.

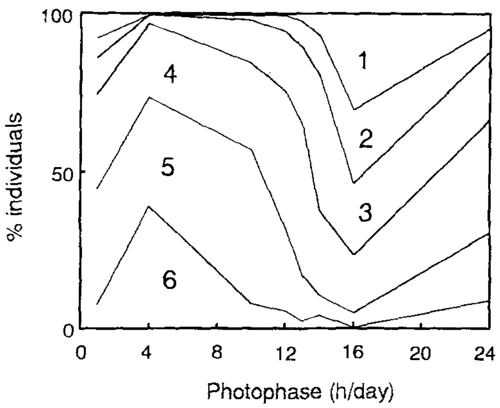

Figure 3. Photoperiodic response curves for the determination of body coloration in the fifth instar nymph of *Plautia stali* at 25 °C. Numerals on the graph indicate grades of body coloration (see fig. 1). n = 57–95.

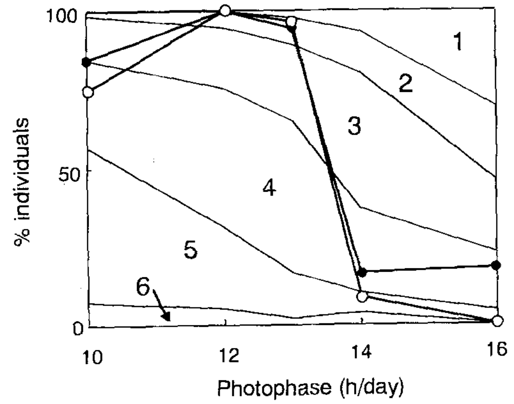

Figure 4. Comparison of the photoperiodic response curves controlling the induction of adult diapause (thick lines) and the determination of body coloration in the fifth instar nymphs (thin lines) in *Plautia stali*. Closed circles, males; open circles, females. Numerals on the graph indicate grades of body coloration (see fig. 1).

Discussion

In *P. stali*, the determination of nymphal body coloration was a quantitative photoperiodic response. Quantitative photoperiodic responses have been known since the 1960s: for example the growth rate of the larvae is quantitatively related to the duration of the photophase in some lepidopterans[1,13,14], and the duration of diapause has also been shown to be quantitatively related to photoperiod in some species[15-19]. In photoperiodic responses which show a sharp threshold length of photo- or scotophase, it has been accepted that the photoperiodic clock judges whether the photo- or scotophase is longer than a threshold value, and the photoperiodic counter successively accumulates long-day or short-day products to a threshold value[20-22]. In photoperiodic responses that vary quantitatively with the photophase duration, however, the clock must measure the photoperiod quantitatively.

We show here that both threshold and quantitative photoperiodic responses exist in a single species, *P. stali*. Unless we assume the existence of different photoperiodic clocks for the two responses, quantitative measurement of photoperiod must also be involved in a threshold response. Recently, it has been shown that the quantitative measurement of photoperiod is involved even in responses that are apparently of the threshold type, by exposing insects during the sensitive period to an unsaturated number of photoperiodic cycles[23-25]. There are some recent theoretical models which propose that the photoperiodic clock measures the photo- or scotophase length in a quantitative manner[2,26,27]. Zaslavski[2] declared that all types of photoperiodic responses are based on quantitative evaluation of photoperiodic information. It is a subject for future study whether the photoperiodic clock in insects generally evaluates the photoperiodic information quantitatively.

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