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Cocoon Enclosure Shifts Photoperiodic Thresholds for Diapause Termination in the Chinese Oak Silkworm, *Antheraea pernyi*

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Abstract

Light transmission through opaque biological coverings is often evaluated using physical measurements; however, such estimates do not necessarily reflect the light environment perceived by organisms. In this study, we examined how cocoon enclosure affects photoperiodic diapause termination in the Chinese oak silkworm, *Antheraea pernyi*. Pupae were maintained under diapause-maintaining conditions (LD 12:12) and exposed daily to an additional 4 h light pulse of defined intensity (0, 0.1, 1, or 10 lux) immediately following the photophase. Naked pupae responded to light pulses of 0.1 lux or higher, whereas cocoon-enclosed pupae required higher intensities to induce diapause termination. At 1 lux, diapause termination was significantly lower in cocoon-enclosed pupae than in naked pupae, while both groups showed similar responses at 10 lux. These results demonstrate a shift in photoperiodic response thresholds associated with cocoon enclosure. Our findings highlight the usefulness of photoperiodic responses as an indicator of light availability and provide a simple and potentially applicable functional framework for assessing light availability within enclosed biological structures.

Keywords: Cocoon enclosure; Diapause; Light intensity; Photoperiodism.

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1. Introduction

Photoperiodism plays a central role in the seasonal regulation of insect development, enabling organisms to synchronize critical life-history events with predictable changes in day length [1,2]. In many insects, photoperiodic information perceived during larval or pupal stages determines whether development proceeds directly or is arrested in a state of diapause [1,3,4]. Because photoperiodic responses often exhibit threshold characteristics, they provide a sensitive biological readout of how environmental light information is perceived and processed.

In several silk moth species, including the Chinese oak silkworm *Antheraea pernyi*

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(Guérin-Méneville), pupal development occurs within a silk cocoon [5]. *Antheraea pernyi* has long been used as a model species in studies of insect photoperiodism owing to its robust and well-characterized photoperiodic diapause responses [4,6,7]. The cocoon provides effective protection against mechanical damage, desiccation, and natural enemies [8-10], but it also attenuates incident light [11-13]. Nevertheless, photoperiodic responses are reliably expressed in cocoon-enclosed pupae, raising the question of how cocoon enclosure influences the light information available to the photoperiodic system.

Early work by Williams *et al*. [12] showed that cocoon-enclosed pupae retain photoperiodic responsiveness despite very low optical transmittance of cocoon material. This led to the hypothesis that the cocoon may redistribute scattered light within the cavity. However, relatively few studies have directly examined how cocoon enclosure affects photoperiodic responses under controlled conditions.

Most previous studies have relied on physical measurements of cocoon optical properties [11-14]. Although such approaches provide useful information, they do not necessarily reflect how light is functionally perceived by the organism. Photoperiodic responses, which integrate light perception and physiological regulation, offer a functional measure of effective light conditions.

In the present study, we used photoperiodic diapause termination in *Antheraea pernyi* as a functional assay to examine how cocoon enclosure affects photoperiodic response thresholds. By comparing naked and cocoon-enclosed pupae, we aimed to characterize how cocoon enclosure modifies photoperiodic responses under defined light conditions.

2. Materials and Methods

2.1. *Insects*

Diapause pupae of the Chinese oak silkworm, *Antheraea pernyi* (Lepidoptera: Saturniidae), were obtained from Silkworm Genetic Resources maintained by the Faculty of Textile Science and Technology, Shinshu University (Ueda, Japan; https://shigen.nig.ac.jp/wildmoth/). Larvae were reared on oak leaves (*Quercus acutissima*) until cocoon formation.

Upon arrival in the laboratory, pupae were maintained at 25 °C under a short-day photoperiod (LD 12:12; 12 h light and 12 h dark, lights on from 10:00 to 22:00) for at least four weeks to ensure stable diapause. In *Antheraea pernyi*, pupal diapause is maintained under a short-day photoperiod (LD 12:12) and can be terminated by exposure to a long-day photoperiod (LD 16:8) [15].

For experiments, pupae were assigned to a naked-pupa group, in which cocoons were carefully removed, and to a cocoon-enclosed group, in which pupae remained within intact cocoons. Both male and female pupae were used. For naked pupae, individuals were assigned so that the proportion of males was approximately equal among groups (40–55 %). For cocoon-enclosed pupae, sex could not be determined before adult emergence, and individuals were therefore selected randomly. After adult emergence, the proportion of

males in the cocoon-enclosed groups was confirmed to be within a similar range (40–55%). Because the objective of this study was to compare photoperiodic responses between naked and cocoon-enclosed pupae rather than to evaluate sex-specific responses, data from both sexes were pooled for analysis.

2.2. *Photoperiodic and light treatments*

Experiments were conducted at 25 °C under LD 12:12 conditions with a light intensity of 100 lux during the photophase. Illumination was provided by white fluorescent lamps (FL10EX-D-Z, Toshiba Corporation, Tokyo, Japan; color temperature 6700 K; color rendering index Ra = 88). The spectral power distribution of the lamp is available from the manufacturer's specifications (https://www.akaricenter.com/chokkan/toshiba/img/3-fl20ssex-bunko-exdexn.jpg).

Pupae were exposed daily to an additional 4-h light pulse immediately following the photophase. The intensity of the additional light pulse was set at 0, 0.1, 1, or 10 lux. After the light pulse, pupae were kept in darkness for the remainder of the scotophase.

The same light regime was applied throughout the experimental period. Each experimental group consisted of 19–23 individuals.

2.3. *Assessment of diapause termination*

Diapause termination was assessed by recording adult emergence daily. Pupae that emerged were considered to have terminated diapause. The incidence of diapause termination was expressed as the cumulative percentage of emerged adults over time for each group.

Observations were continued for 60 days after the start of light treatments. Individuals that did not emerge during this period were subsequently transferred to long-day conditions (LD 16:8, 100 lux), under which all individuals eventually emerged.

2.4. *Statistical analysis*

Time courses of diapause termination were compared among groups using Kaplan–Meier analysis followed by log-rank tests. Comparisons between naked and cocoon-enclosed pupae at each light intensity were performed using this method. Final diapause termination rates (day 60) were compared using Fisher's exact test.

All analyses were conducted using R (version 4.5.3). Differences were considered significant at $p < 0.05$.

3. Results

We compared diapause termination between naked pupae and pupae enclosed within intact cocoons of *Antheraea pernyi* under controlled light conditions (Fig. 1). Pupae were exposed daily to an additional 4 h light pulse of defined intensity (0, 0.1, 1, or 10 lux) following the

photophase (Fig. 1A).

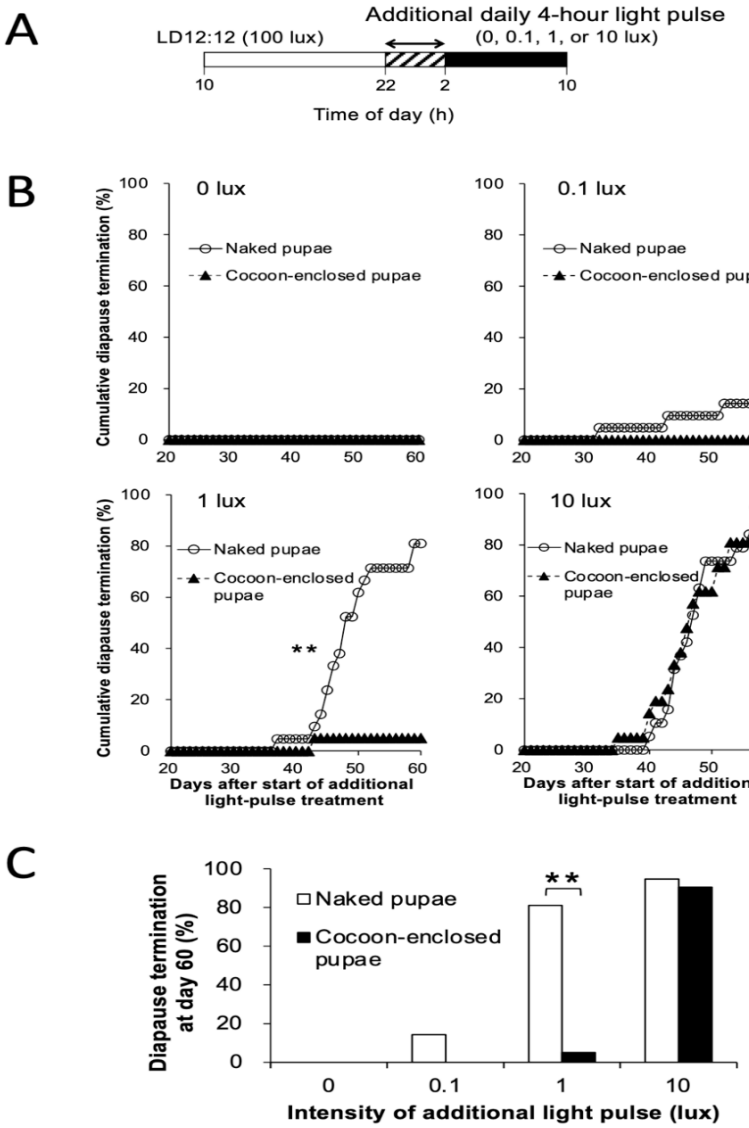

Fig. 1. Photoperiodic assay of diapause termination in *Antheraea pernyi*. (A) Schematic representation of the light conditions. Pupae were maintained under LD 12:12 (100 lux during the photophase), followed by a daily 4 h light pulse at 0, 0.1, 1, or 10 lux. (B) Time course of diapause termination in naked pupae and cocoon-enclosed pupae under each light condition. Diapause termination was assessed as adult eclosion and is expressed as the cumulative percentage of emerged adults. Differences between groups were analyzed using Kaplan–Meier analysis with the log-rank test. Asterisks indicate significant differences ($p < 0.01$). (C) Final incidence of diapause termination at day 60 under each light condition. Differences between groups were analyzed using Fisher's exact test. Asterisks indicate significant differences ($p < 0.01$). Sample sizes were 19–23 individuals per group.

Diapause termination responses differed between groups across light intensities (Fig. 1B). In naked pupae, a slight response was observed at 0.1 lux, whereas cocoon-enclosed pupae showed no detectable response at this intensity. At 1 lux, diapause termination occurred in both groups; however, the cumulative incidence was significantly lower in cocoon-enclosed pupae than in naked pupae (log-rank test, $p < 0.01$). At 10 lux, diapause termination progressed rapidly and reached similarly high levels in both groups. In both naked and cocoon-enclosed pupae, adult emergence occurred earlier as light intensity increased, indicating that the diapause termination period was shorter under higher light intensities. No diapause termination was observed in either group under the 0-lux condition.

Consistent with the time-course analysis, the final incidence at day 60 indicated a shift in the response threshold between naked and cocoon-enclosed pupae (Fig. 1C). Naked pupae responded at lower light intensities than cocoon-enclosed pupae, with a significant difference at 1 lux (Fisher's exact test, $p < 0.01$), whereas no difference was observed at 10 lux. All individuals that had not emerged by day 60 subsequently emerged under long-day conditions.

4. Discussion

In the present study, we examined how cocoon enclosure affects photoperiodic diapause termination in *Antheraea pernyi*. By comparing naked pupae with pupae enclosed within intact cocoons, we found that cocoon enclosure was associated with a shift in the photoperiodic response threshold, such that higher light intensities were required to induce diapause termination in cocoon-enclosed pupae. Although cocoon enclosure is expected to reduce light availability, the present results show that this effect is expressed as a shift in the photoperiodic response threshold rather than a simple attenuation of response magnitude.

No difference between naked and cocoon-enclosed pupae was observed at 10 lux, where both groups exhibited similar diapause termination patterns, whereas clear differences emerged at lower light intensity (1 lux). This pattern supports an intensity-dependent shift in photoperiodic response rather than a general effect of cocoon removal. In addition, increasing light intensity accelerated diapause termination in both naked and cocoon-enclosed pupae, indicating that light intensity affects not only the incidence of diapause termination but also its timing.

Light intensity in the present study was expressed in lux, which is based on human photopic sensitivity and does not directly correspond to the spectral sensitivity of insect photoreceptors. Therefore, the present results should be interpreted within the defined spectral conditions rather than as absolute intensity thresholds. Direct measurement of internal light intensity would be technically informative; however, such measurements do not necessarily reflect the light environment perceived by the organism.

Previous studies have shown that cocoons attenuate incident light while still permitting photoperiodic responses in enclosed pupae [12]. The present study extends these findings by demonstrating that cocoon enclosure shifts the response threshold of the photoperiodic

system, without substantially altering the overall response pattern.

Although the present study was conducted under a limited set of light intensities and spectral conditions, these results demonstrate that photoperiodic responses can serve as a simple functional indicator of light availability within intact biological structures.

5. Conclusion

Cocoon enclosure shifts the photoperiodic response threshold for diapause termination in *Antheraea pernyi*, requiring higher light intensities to induce responses. These findings indicate that cocoon enclosure alters the effective light environment perceived by the organism. Photoperiodic responses provide a useful functional indicator for assessing light availability within enclosed biological structures.

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