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# Exposing Larvae to Night Lighting Inhibits Embryonic Diapause in the Next Generation in the Domestic Silkworm, *Bombyx mori*

A. Iwamoto<sup>1</sup>, Y. Egi<sup>1</sup>, K. Sakamoto<sup>1,2\*</sup>

<sup>1</sup>Graduate School of Agricultural Science, Kobe University, 1-1 Rokkodai-cho, Nada-ku, Kobe 657-8501, Japan

<sup>2</sup>Biosignal Research Center, Kobe University, 1-1 Rokkodai-cho, Nada-ku, Kobe 657-8501, Japan

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#### Abstract

The domestic silkworm ( $Bombyx\ mori$ ) is reared under controlled environments. Therefore, silkworms may be exposed at night to light used by humans working or living nearby. Artificial light at night (LAN) disturbs natural lighting cycles and negatively affects insect physiology, behavior, and ecology in the field. However, the effects of dim LAN on domestic silkworms have not been assessed in detail. The present study investigated the effects of night lighting on diapause regulation in  $Bombyx\ mori$ , with a focus on light sensitivity. The silkworm larvae were exposed to LAN at intensity varying from 0.1-100 lux under a short-day photoperiod that should induce diapauses, and then the incidence of diapause in eggs laid by resultant moths was evaluated. The incidence of diapause was 100% in control (without night lighting) silkworms, but the incidence in those exposed to LAN  $\geq 0.1$  lux was < 2%. These results showed that exposing larvae to night lighting at 0.1 lux under a short-day photoperiod substantially inhibited diapause induction in the next generation.

Keywords: Bombyx mori; Embryonic diapause; Light sensitivity; Night lighting.

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#### 1. Introduction

The domestic silkworm *Bombyx mori* L. (Lepidoptera: Bombycidae) is a major component of the sericulture industry and an economically important resource organism. Therefore, information about breeding silkworms is valuable.

For silkworm rearing in sericulture, diapause eggs that can be stored at low temperatures for a long time (several months) are usually used. Diapause is the hormonally regulated arrest of insect development or reproduction to cope with harsh environmental conditions [1,2]. Silkworms exhibit maternally controlled embryonic diapause. Bivoltine silkworms are destined to produce diapause or non-diapause eggs depending on environmental cues such as temperature and photoperiod (length of day) during the egg and larval stages [3,4].

 $<sup>^* \</sup>textit{Corresponding author}: \underline{ksakamoto@diamond.kobe-u.ac.jp}$ 

Artificial light at night (LAN), derived from streetlights and other illuminated structures, deprives nature of true darkness at night, disturbs natural lighting cycles [5,6], and negatively affects insect physiology, behavior, and ecology in the field [7-9]. Artificial LAN also alters the daily and seasonal activity patterns of wild insects [10,11]. Actually, LAN has been shown to affect season-dependent diapause regulation in several insect species, including mosquitoes, flies, and moths [12-15].

As silkworms are reared under human-controlled environments, they may be exposed at night to light used by humans working or living nearby. However, the effects of exposure to dim LAN on silkworm diapause have not been investigated in detail. Therefore, the present study aimed to determine the effects of dim LAN on diapause regulation in silkworms, with a focus on light sensitivity.

## 2. Materials and Methods

Silkworm eggs of the p50 strain provided by the National Bio-Resource Project (NBRP) of the Ministry of Education, Science, Sports and Culture of Japan were incubated at 25 °C under continuous darkness. Hatched larvae were reared at 25 °C under a daily 12 h light-12 h dark (day-night) cycle (short days) until they became adult moths and laid eggs. Daytime light intensity was adjusted to 100 lux at the level of the animals. The intensity of nighttime illumination was adjusted to 0, 0.1, 1, 5, or 100 lux from hatching until the larvae started spinning cocoons. The source of illumination was a white fluorescent lamp (FL10EX-D-Z, Toshiba Corporation, Tokyo, Japan). The light intensity was measured using a digital illuminance meter (LX-1181, MOTHERTOOL Co. Ltd., Ueda, Japan). The larvae were fed with the Silkmate PS Artificial Diet (Nihonnousan Kogyo Co. Ltd., Yokohama, Japan).

The incidence of diapause, defined as the ratio (%) of female moths (n = 33–74) that laid diapause eggs [16], was compared among experimental groups using Fisher's exact test with Benjamini-Hochberg multiple testing correction. Values with P < 0.05 were considered significant.

#### 3. Results and Discussion

The diapause incidence of silkworms exposed to LAN of 0 lux (control) was 100 %, but exposure to LAN  $\geq$  0.1 lux reduced the incidence to < 2 % (Fig. 1).

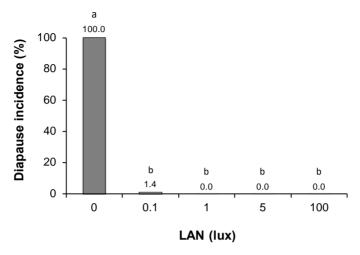


Fig. 1. Effects of dim light at night (LAN) on diapause regulation in silkworms. Superscript letters indicate significant differences among groups (P < 0.01).

Exposing silkworm larvae to dim LAN under a short-day photoperiod inhibited embryonic diapause in the next generation like a long-day photoperiod, which induces silkworms to produce non-diapause eggs. Previous studies have also indicated that LAN under short days potentially inhibits diapause in other insects [12-15]. It is likely that LAN lengthened perceived day length in insects.

The present study showed that exposure to 0.1 lux LAN obviously reduced the incidence of diapause. The intensity of nighttime illumination during the larval stage should be carefully considered to avoid inducing the production of non-diapause eggs by silkworms that are specifically geared to produce diapause eggs.

Others have found that rearing silkworm larvae under continuous light increases larval duration, as well as pupal and cocoon shell weight, more than when reared under a daily light-dark cycle [17,18]. Therefore, exposing larvae to LAN might be beneficial in silk production in some respects.

### 4. Conclusion

The present study found that exposing silkworm larvae to dim LAN under short days inhibits embryonic diapause in the next generation. Silkworm larvae were sensitive to nighttime light intensity of 0.1 lux. Nighttime illumination intensity during the larval stage should be carefully considered to avoid the inhibitory effects of excessive LAN when rearing silkworms to obtain diapause eggs for breeding or storage.

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