Effects of photoperiod on the induction of egg diapause of the wild silkworm, *Bombyx mandarina* Moore

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1. INTRODUCTION

The wild silkworm, Bombyx mandarina, inhabits China, Korea and Japan (1). Copulation between B. mandarina and the domestic silkworm, B. mori, is known to be possible, and the resultant progenies are never sterile (e.g., 2-5). Therefore, it is certain that B. mandarina and B. mori are closely related species. Sasaki (6) even insisted that B. mandarina was derived from B. mori.

There are very few studies on egg diapause and the life cycle of B. mandarina. Ohba (7) reported that the duration of hatching from diapausing egg and the pupal period of B. mandarina were longer and more variable than those of B. mori. Ohmura (8), who reared B. mandarina in the laboratory and observed the same phenomena, inferred that B. mandarina might have a complex voltinism consisting of univoltine, bivoltine, trivoltine and tetravoltine individuals: the majority of individuals within the same population trivoltine, some of them bivoltine and a few univoltine and tetravoltine. However, environmental factors which affect the induction of egg diapause and regulate the life cycle of B. mandarina are still unknown.

In this paper I will describe effects of photoperiod on the induction of egg diapause of B. mandarina and make a comparison between B. mandarina and B. mori.

2. MATERIALS AND METHODS

Four geographical strains of B. mandarina were used in this experiment. One of them was collected in China and the others in Japan. Collecting sites are shown in Table 1.

Table 1. Collecting sites of geographical strains of B. mandarina examined in this study

Geographical Strain	*Collecting site*	*Latitude*
Sakado	*Sakado, Saitama, Japan*	*36.0°N*
To-shima	*To-shima Island, Tokyo, Japan*	*34.5°N*
Kozu-shima	*Kozu-shima Island, Tokyo, Japan*	*34.2°N*
Hangzhou	*Hangzhou, Zhejiang, China*	*30.2°N*

Each strain was kept in an insect rearing chamber (Shimadzu Co., Ltd.) from egg incubation until adult emergence. Four photoperiodic regimes were established for this experiment. Two of them were constant photoperiods, long-day (15L9D) and short-day (12L12D). The others were photoperiodic changes, long-day (15L9D) to short-day (12L12D) and vice versa. In the case of photoperiodic changes, the 3rd instar larvae just after the 2nd larval ecdysis were transferred from one photoperiod to the other. Temperature was kept at 25°C during this experiment. Larvae were reared on mulberry leaves.

3. RESULTS

Photoperiodic responses of each strain are shown in Table 2. In the Sakado strain, three batches of eggs (A, B and C in Table 2) were prepared and the results from each batch are shown separately. In each of the other strains, however, two batches of eggs were prepared and the combined results from these two are shown; this was because, in these strains, the number of female moths had decreased owing to low hatchability of eggs and/or outbreak of disease during the larval period.

Every female moth of the Sakado strain produced non-diapausing eggs under long-day (15L9D) photoperiod, and the % of these females increased as follows: 12L12D-15L9D < 15L9D-12L12D < 12L12D. The result that more female moths produced diapausing eggs under short-day than under long-day strongly suggests that the photoperiodic response curve of the Sakado strain is a long-day type. Also, from the similarity in the % of female moths producing diapausing eggs between 12L12D-15L9D and 15L9D and between 15L9D-12L12D and 12L12D, respectively, it is concluded that in this strain the most sensitive stage to photoperiod exists in a certain developmental stage following the 2nd larval ecdysis.

Table 2. Effects of photoperiod on the induction of egg diapause of geographical strains of B. mandarina

Geographical strain	*% of female moths producing diapausing eggs (No. of female moths examined)*			
	Photoperiodic regime			
	15L9D	*12L12D*	*15L9D -12L12D*	*12L12D -15L9D*
Japan				
Sakado A	*0.0(36)*	*100.0(37)*	*74.0(39)*	*35.5(31)*
B	*0.0(13)*	*90.0(10)*	*86.0(14)*	*25.0(8)*
C	*0.0(23)*	*77.8(27)*	*73.0(15)*	*0.0(25)*
To-shima	*0.0(12)*	*100.0(12)*	*57.1(7)*	*0.0(10)*
Kozu-shima	*0.0(21)*	*57.9(18)*	*58.3(12)*	*0.0(18)*
China				
Hangzhou	*0.0(8)*	*87.5(8)*	*0.0(4)*	*87.5(8)*

Photoperiodic responses of the To-shima strain and Kozushima strain were similar to the Sakado strain. Therefore, it is also suggested that these strains have long-day responses to photoperiod and that the most sensitive stages to photoperiod exist in certain developmental stages following the 2nd larval ecdysis. In the case of the Kozu-shima strain, however, the % of female moths producing diapausing eggs under 12L12D was an intermediate value (57.9%). This may reflect the fact that the critical photoperiod of the Kozu-shima strain is shorter than the other strains.

Although the Chinese (Hangzhou) strain is also suggested to have a long-day type of response to photoperiod, the most sensitive stage to photoperiod of this strain is different from that of the Japanese strains. In this strain, apparently, the most sensitive stage to photoperiod is in a certain stage prior to the 2nd larval ecdysis.

4. DISCUSSION

The diapause-inducing photoperiodic responses of B. mori (9-13) and B. mandarina are summarized in Table 3. In B. mori there are two evident relationships between voltinism and photoperiodic response. First of all, as voltinism increases, the temperature during the incubation period of the egg which permits B. mori to respond to photoperiod rises. Univoltine races show weak photoperiodic responses only when temperature

Table 3. Classification of photoperiodic responses for the induction of egg diapause of B. mori and B. mandarina

Race or strain	Food	Temperature during egg incubation	Type of photoperiodic response (Most sensitive stage)
B. mori			
Univoltine race	Mulberry leaf	15°C	Short-day type (Egg)
Bivoltine race	Mulberry leaf	20°C	Short-day type (Egg-3rd instar) Long-day type (4th instar-pupa)
	Artificial diet	16°C, 25°C	Long-day type (3rd and 4th instars)
Tropical race			
Ringetsu -type	Mulberry leaf	25°C	Short-day type (Egg-2nd instar) Long-day type (3rd instar-pupa)
Annam -type	Mulberry leaf	25°C	Long-day type (3rd instar-pupa)
B. mandarina			
Japanese strain	Mulberry leaf	25°C	Long-day type (3rd instar-pupa)
Chinese strain	Mulberry leaf	25°C	Long-day type (Egg-2nd instar)

during incubation is 15°C (9). Bivoltine races show striking photoperiodic responses when temperature during incubation ranges between 15°C and 25°C (9). Tropical multivoltine races show photoperiodic response even when temperature during incubation is 25°C (12-13).

Second, as voltinism increases, the type of photoperiodic response changes from a short-day response to a long-day response and, at the same time, the most sensitive stage to photoperiod moves from earlier developmental stages to later stages. Univoltine races show weak short-day responses during the egg incubation period (9). Bivoltine races show strong short-day responses during this period and earlier larval stages, while they show weak long-day responses during the later larval stages and pupal period (9). Photoperiodic responses of tropical multivoltine races are divided into two groups (12). "Ringetsu-type races" show weak short-day responses during the egg incubation period and earlier larval stages, while they show strong long-day responses during later larval stages and pupal period. "Annam-type races" show strong long-day responses during later larval stages and pupal period.

It is well known that photoperiodic response of insects can be modified by dietary conditions (see, e.g., 14-17). In the case of bivoltine races of *B. mori*, expression of a long-day type of response during the third and fourth larval instars becomes predominant (10-11).

B. mandarina, like tropical multivoltine races of *B. mori*, shows a long-day type of response when temperature during incubation is 25°C. There is, however, a difference in the most sensitive stage to photoperiod between the three Japanese strains and one Chinese strain as shown by the results. There also is a difference in chromosome number between these strains. Although chromosome number of the Japanese strain is $n=27$ (2), that of the Chinese strain is $n=28$, the same as that of *B. mori* (18-19). Therefore, the Japanese strain is thought to be genetically and ecologically independent from the Chinese strain.

In many reviews and books, *B. mori* is described as a short-day insect (see, e.g., 14-17). However, recent studies on tropical races of *B. mori* revealed that these races had long-day responses to photoperiod (12-13). This paper has demonstrated that the closely related species, *B. mandarina*, also has long-day responses. From these facts, I propose that our earlier conclusion that *B. mori* is a short-day insect should be revised.

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