Effects of Photoperiod on the Induction of Egg Diapause of Tropical Races of the Domestic Silkworm, *Bombyx mori*, and the Wild Silkworm, *B. mandarina*

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Introduction

The wild silkworm, *Bombyx mandarina*, inhabits China, Korea and Japan[15]. Copulation between *B. mandarina* and the domestic silkworm, *B. mori*, is known to be possible, and the resultant progenies are never sterile[1,4]. Therefore, it is certain that *B. mandarina* and *B. mori* are closely related species. Sasaki[10] even insisted that *B. mandarina* be derived from *B. mori*.

There are very few studies on egg diapause and the life cycle of *B. mandarina*. Ohmura[9] inferred that *B. mandarina* might have a complex voltinism which consists of univoltine, bivoltine, trivoltine and tetravoltine individuals: the majority of individuals within the same population are 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.

On the other hand, effects of environmental factors (especially photoperiod) on the induction of egg diapause of tropical races of *B. mori* are not sufficiently investigated, as compared with uniboltine and bivoltine races[8,12-13].

In this paper I will describe effects of photoperiod on *B. mandarina* and tropical races of *B. mori*, and make a comparison between *B. mandarina* and *B. mori*.

Materials and methods

Eight tropical races of *B. mori* and four geographical races of *B. mandarina* were used in this experiment. Origins and years of introduction to Japan of tropical races are shown in Table 1 and sites and years of collecting *B. mandarina* are shown in Table 2.

Each race was kept in an insect rearing chamber (Shimizu 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

Table 1. Origins and years of introduction into Japan of tropical races of *B. mori* examined in this study

Tropical race	Origin	Year of introduction
Cambodge (fixed race)	Indo-China	1935
Annam	Indo-China	1935
Cambodge	Indo-China	1935
Pure Mysore	India	Unknown
Mysore	India	1935
Ringetsu	China (Guangdong)	1934
Br 9	Thailand	1983
Nk 4	Thailand	1983

Table 2. Sites and years of collecting geographical races of *B. mandarina* examined in this study

Geographical race	Collecting site	Latitude	Collecting year
Sakado	Sakado, Saitama, Japan	36.0°N	1982
To-shima	To-shima Island, Tokyo, Japan	34.5°N	1985
Kozu-shima	Kozu-shima Island, Tokyo, Japan	34.2°N	1985
Hangzhou	Hangzhou, Zhejiang, China	30.2°N	1981

Table 3. Effects of photoperiod on the induction of egg diapause of tropical races of *B. mori*

Tropical race	Percentage of female moths producing diapausing eggs			
	Photoperiodic regime			
	15L9D	12L12D	15L9D -12L12D	12L12D -15L9D
Cambodge (fixed race)	26.6(32)*	57.6(46)	89.0(41)	14.1(32)
Annam	23.0(37)	62.2(37)	60.0(55)	15.4(39)
Cambodge	12.5(36)	74.5(47)	71.3(40)	24.3(35)
Pure Mysore	38.7(31)	97.5(20)	95.8(24)	72.2(27)
Mysore	85.0(40)	92.2(32)	98.8(40)	41.7(42)
Ringetsu	34.9(43)	36.7(60)	100.0(47)	3.8(53)
Br 9	23.1(39)	41.9(31)	38.7(31)	31.7(41)
Nk 4	0.0(30)	6.3(24)	15.5(29)	0.0(30)

* Numerals in parentheses indicate the number of female moths examined.

Table 4. Effects of photoperiod on the induction of egg diapause of geographical races of *B. mandarina*

Geographical race	Percentage of female moths producing diapausing eggs			
	Photoperiodic regime			
	15L9D	12L12D	15L9D -12L12D	12L12D -15L9D
Japan				
Sakado	0.0(72)*	90.5(74)	76.3(68)	20.3(64)
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)

* See the footnote of Table 3.

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.

Photoperiodic responses of tropical races of *B. mori*

Table 3 shows photoperiodic responses of each tropical race. In all races, more female moths produced diapausing eggs under short-day (12L12D) photoperiod than long-day (15L9D). From this result, it is suggested that the tropical races have long-day responses to photoperiod.

In Cambodge (fixed race), Mysore and Ringetsu, female moths produced more diapausing eggs under 15L9D-12L12D than 12L12D and more non-diapausing eggs under 12L12D-15L9D than 15L9D. Especially in Ringetsu, it was revealed that photoperiodic response before the 2nd larval ecdysis was a short-day type and after that a long-day type[5,6]. Therefore, Cambodge (fixed race) and Mysore would probably have the same type of photoperiodic response. This type of response is termed "Ringetsu-type" in this paper.

In the other races, it is obvious that the most sensitive stages to photoperiod exist in certain developmental stages following the 2nd larvae ecdysis. This, another type of response is termed "Annam-type".

Except for Br 9 and Nk 4, which were recently introduced from Thailand, the tropical races showed strong sensitivity to photoperiod. This result is seemed to reflect that sensitivity to photoperiod was enhanced by adaptation to the rearing conditions in Japan for many years.

Photoperiodic responses of *B. mandarina*

Photoperiodic responses of geographic races are shown in Table 4. In all races, more female moths produced diapausing eggs under short-day than long-day. Accordingly, it is suggested that *B. mandarina* is a long-day insect.

In Japanese races, from the similarity in the percentage of female moths producing diapausing eggs between 12L12D-15L9D and 15L9D and between 15L9D-12L12D and 12L12D, respectively, it is concluded that the most sensitive stages to photoperiod exist in certain developmental stages following the 2nd larval ecdysis. In the case of the Kozu-shima race, however, the percentage 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 that of the other strains.

Although the Chinese (Hangzhou) race is also suggested to have a long-day type of response to photoperiod, the most sensitive stage to photoperiod of this race is in a certain stage prior to the 2nd larval ecdysis.

Comparison of photoperiodic responses between *B. mori* and *B. mandarina*

The diapause-inducing photoperiodic responses of *B. mori*[5,6,8,12,13] and *B. mandarina* are summarized in Table 5. In *B. mori* there are two evident relationships between voltinism and photoperiodic response. First, 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 during incubation is 15°C[8]. Bivoltine races show striking photoperiodic responses when temperature during incubation ranges between 15°C and 25°C[8]. As revealed in this paper, tropical multivoltine races show photoperiodic response even when temperature during incubation is 25°C.

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[8]. Bivoltine races show strong short-day responses during this period and earlier larval stages, while they show weak

Table 5. Classification of photoperiodic responses inducing egg diapause in *B. mori* and *B. mandarina*

Race	Food	Temperature during egg incubation	Type of photoperiodic response	The 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-4th instar
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 race	Mulberry leaf	25°C	Long-day type	3rd instar-pupa
Chinese race	Mulberry leaf	25°C	Long-day type	Egg-2nd instar

long-day responses during the later larval stages and the pupal period[8]. Photoperiodic responses of tropical multivoltine races are divided into two groups. "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 the later larval stages and the pupal period. "Annam-type races" show strong long-day responses during the later larval stages and the pupal period.

It is well known that photoperiodic response of insects can be modified by dietary conditions[2,3,11,14]. 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[12,13].

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 races and one Chinese race. There also is a difference in chromosome number between these geographic races. Although chromosome number of the Japanese race is $n=27$[4], that of the Chinese race is $n=28$[7], the same as that of *B. mori*[4]. Therefore, the Japanese race is thought to be genetically and ecologically independent from the Chinese race.

In many reviews and books, *B. mori* is described as a short-day insect[2,3,11,14]. However, this paper has demonstrated that tropical races of *B. mori* and the closely related species, *B. mandarina*, have long-day responses. From these facts, I propose that our earlier conclusion that *B. mori* is a short-day insect be revised.

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