# Effects of photoperiod on the duration of pupal stage of the wild silkworm, *Bombyx mandarina* Moore

Jun Kobayashi

Department of Insect Genetics and Breeding National Institute of Sericultural and Entomological Science Tsukuba, Ibaraki 305, Japan

## 1. INTRODUCTION

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The wild silkworm, <u>Bombyx</u> <u>mandarina</u>, and the domestic silkworm, <u>B</u>. <u>mori</u>, are closely related species. Coupulation between them is known to be possible, and the resultant progenies are never sterile (e.g., 1-4).

Only a little is known about the life cycle of <u>B</u>. <u>man-darina</u>. Ohba (5) and Ohmura (6) reported that the duration of hatching from diapausing egg and the duration of pupal stage of <u>B</u>. <u>mandarina</u> were longer and more variable than those of <u>B</u>. <u>mori</u>. However, environmental factors which affect the life cycle of <u>B</u>. <u>mandarina</u> are still unknown. Recently I reported that female moths of <u>B</u>. <u>mandarina</u> produced diapausing eggs when they were reared under short-day photoperiod during the larval stage, like the tropical races of <u>B</u>. <u>mori</u> (7).

In this paper I will describe effects of photoperiod on the pupal duration of <u>B</u>. <u>mandarina</u> and the relationship between the pupal duration and the induction of egg diapause.

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Table 1. Sites of collecting geographical strains of <u>B</u> . <u>mandarina</u> examined in this study					
Geographical strain	Collecting site	Latitude			
Sakado To-shima Kozu-shima Hangzhou	Sakado, Saitama, Japan To-shima Island, Tokyo, Japan Kozu-shima Island, Tokyo, Japan Hangzhou, Zhejiang, China	36.0°N 34.5°N 34.2°N 30.2°N			

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## 2. MATERIALS AND METHODS

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

Each strain was kept in an insect rearing chamber (Shimazu 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 <u>vice versa</u>. 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 Fig. 1 and Table 2. The pupal duration of the Sakado strain varied with photoperiodic regimes (Fig. 1a). In both male and female, the means of pupal duration were the shortest (18-19 days) under long-day (15L9D) photoperiod and became longer (25-27 days) under short-day (12L12D) (Table 2). Furthermore, under the photoperiodic changes (15L9D-12L12D and 12L12D-15L9D) the means became the longest values (28-30 days). Because the distributions of the pupal duration were very wide (13-41 days) under these changes, the standard deviations also became larger (4-9 days).

Photoperiodic responses of the To-shima strain and Kozushima strain were different from the Sakado strain. In these strains, most of pupal durations were longer than 20 days and the distributions of the pupal duration were relatively wide under all the photoperiodic regimes (Fig. 1b-c). Therefore, the means and the standard deviations of the pupal duration under each photoperiodic regimes were as large as those of the Sakado strain under the photoperiodic changes (Table 2).

Although the Chinese strain, like the Sakado strain, showed shorter mean of the pupal duration (17 days) under 15L9D and longer mean (22 days) under 12L12D, this strain showed the shortest mean (15 days) under 15L9D-12L12D and longer mean (22-23 days) under 12L12D-15L9D (Table 2). These results strongly suggest that the most sensitive stage to photoperiod of this strain is in a certain stage prior to the 2nd larval ecdysis. In this strain there was no individual whose pupal duration was more than 30 days (Fig. 1d).

In Fig. 2, the relathionship between the pupal duration of females and the induction of egg diapause was summarized. Under 15L9D-12L12D, female moths of the Sakado strain experienced shorter pupal durations produced non-diapausing eggs and those experienced longer pupal durations produced diapausing eggs (Fig. 2a). Under 12L12D-15L9D, however, most of female moths experienced longer pupal durations produced nondiapausing eggs. From this disagreement between the pupal duration and the induction of egg diapause, it is obvious that these two developmental phenomena are independently controlled by photoperiod.

In the To-shima strain and Kozu-shima strain, because the pupal duration was not affected by photoperiod, there was no obvious relationship between the pupal period and the induction of egg diapause (Fig. 2b-c).

In the Chinese strain, female moths experienced shorter pupal durations (<19 days) produced non-diapausing egg and those experienced longer pupal durations (>19 days) produced diapausing eggs (Fig. 2d). This agreement between these two developmental phenomena was considered as a reflection of the coincidence of their most sensitive stages to photoperiod.

## 4. DISCUSSION

It is well known that the wild silkworm, <u>Bombyx man-</u><u>darina</u>, hibernates at the egg stage and there is no observation on the overwintering at the pupal stage in the field. Ohba (8) reported that all the pupae collected in September had become moths before December. In this study, although the Sakado strain and the Chinese strain showed longer pupal durations under 12L12D than under 15L9D, the longest duration under 12L12D was about 30 days and seemed not to be sufficient for overwintering. Therefore, it is strongly suggested that the pupal stage is not the overwintering stage of <u>Bombyx mandarina</u>. If there are some ecological roles in the long pupal duration, they must be played from spring to autumn.

Ohmura (6), who reared <u>B</u>. <u>mandarina</u> in the laboratory and observed its long and variable pupal duration, inferred that <u>B</u>. <u>mandarina</u> might have a complex voltinism: the majority of individual within the same population trivoltine, some of them bivoltine and a few univoltine and tetravoltine. He thought that this complex voltinism were mainly caused by the long and variable pupal duration. In fact, any time from June to Sep-





Fig. 1. Effects of Photoperiod on the pupal duration of geographical strains of <u>B</u>. <u>mandarina</u>

Googra-	Pupal duration (mean±SD)					
phical strain	Photoperiodic regime					
	15L9D	12L12D	-12L12D	-15L9D		
Japan						
Sakado	319.2±1.8(54)	27.3±2.1(35)	29.6±5.6(44)	28.0±3.9(41)		
	Ŷ18.4±1.6(36)	25.0±2.4(40)	29.3±8.6(41)	29.4±5.9(32)		
To-	\$26.1±3.7(31)	27.0±3.6(49)	28.8±3.8(35)	27.8±3.3(20)		
shima	♀27.2±4.5(13)	29.6±3.0(13)	34.0±3.8( 9)	27.5±3.7(11)		
Kozu-	\$25.4±3.1(59)	27.4±2.6(52)	28.4±2.8(53)	25.7±3.3(61)		
shima	♀28.1±5.8(28)	28.9±3.0(26)	32.5±5.1(20)	26.8±4.3(21)		
China						
Hangzhou	317.1±1.1(15)	22.1±2.0(15)	14.7±1.0( 9)	22.0±6.4(8)		
	♀17.1±2.4(15)	21.9±3.4(13)	15.2±1.2( 6)	23.0±3.8(11)		

Table 2. Effects of photoperiod on the pupal duration of geographical strains of <u>B</u>. <u>mandarina</u>

Numerals in parentheses indicate the number of pupae examined.







Fig. 2. The relationship between the pupal duration of females and the induction of egg diapause

tember, almost all stages of <u>B</u>. <u>mandarina</u> can be observed in a mulberry field. This fact is one of proofs that the population of <u>B</u>. <u>mandarina</u> consists of different generations.

Intrapopulation variations in insect seasonal cycles are frequently observed in the field and some of them appear especially important in spreading over time the risks involved with terminating dormancy and initiating growth, development, and reproduction in an unpredictable environment (9). Therefore, those long and variable pupal durations in the To-shima strain and Kozu-shima strain (Fig. 1b-c) might reflect unpredictable, environmental changes in these islands. The Sakado strain also exihibited long and variable pupal durations under the photoperiodic changes (Fig. 1a). In this strain the sudden change in photoperiod might be recieved as a unpredictable environment.

In this paper, it was revealed that there were differences in effects of photoperiod to the pupal duration among geographical strains of <u>B</u>. <u>mandarina</u> and that, at least in the Japanese strains, the pupal duration and the induction of egg diapause were independent phenomena. As yet, only little is known about the life cycle of <u>B</u>. <u>mandarina</u> in the field. Thus, for further study of the functions and adaptive value of the long and variable pupal duration, it will be necessary to investigate the wild population in detail.

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