EXISTENCE OF SEXUAL DIFFERENCE IN THE EMERGENCE RHYTHM OF THE SILKWORM MOTHS, *BOMBYX MORI* L.

By

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As reported in the previous paper by the senior author (KOBAYASHI & KOYAMA, 1974), the daily population rhythm of emergence in the silkworm moths, *Bombyx mori* L. appears with bimodal peaks when the pupae are subjected to short-day illumination such as 3 L 21 D, whereas it is generally unimodal per day under natural condition or long-day regimes. However, the reason remains unsolved why each rhythmic peak is divided into two ones in such a condition.

Tried to solve the question, the authors carried out the experiments, in which the silkworm pupae were exposed to various photoperiods, and have come to the conclusion that it is originated by the sexual difference in photoperiodic entrainment of the pupae.

Before going further the authors desire to express their hearty thanks to the members of the Biological Laboratory who assisted the work.

MATERIAL AND METHOD

The pupae of the three hybrids of the silkworm were used for the experiments. About 50 ~ 100 individuals of the male and the female pupae were exposed to the following photoperiodic conditions, respectively.

Shungetsu × *Hosho* (normal rearing with mulberry leaves)

24 D, 10 L 14 D (10 hours-light : 14 hours-dark), 8 L 16 D, 6 L 18 D, 4 L 20 D, 2 L 22 D, 1 L 23 D, 0 : 30 L 23 : 30 D (30 min.-light : 23 hours and 30 min.-dark), 0 : 15 L 23 : 45 D, 0 : 05 L 23 : 55 D, 0 : 01 L 23 : 59 D

Shinki × *Ryoho* (asceptic rearing with artificial diet)

Ryoho × *Shinki* (ditto)

9L15D, 6L18D, 3L21D

The light intensity was 100 lux at the pupal surface. The room temperature was 25 ± 0.5°C with R. H. 75 ± 5 %. The emerged moths were counted every two hours using dim red light.

RESULTS

1. *Shungetsu* × *Hosho*

24D (Fig. 1)

When the pupae were confined to a constant darkness, three endogenous peaks appeared in emergence feature. The interval of free-running rhythm, however, varied 18∼28 hours (Fig. 1A). As shown in Fig. 1B, the first peak was mainly consisted of the male moths, and the second and the third peaks consisted of the female moths, respectively.

The phenomenon in which occurred two peaks per day was not recognizable, though the eclosion time was faster about one day in the male than in the female.

10L14D (Fig 2)

The emergence feature was evidently rhythmical, being entrained by the

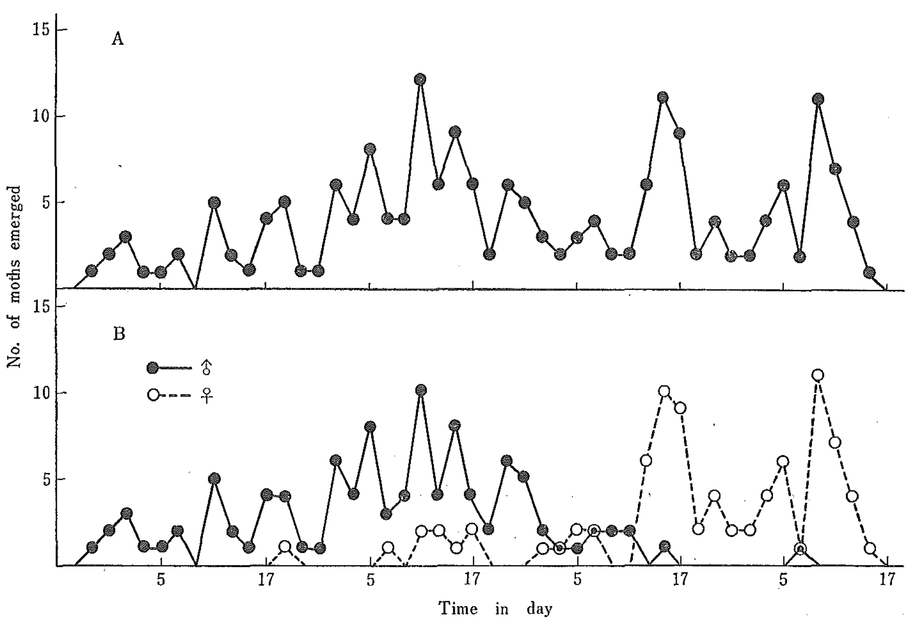

Fig. 1 Emergence feature in *Shungetsu* × *Hosho* under constant darkness.

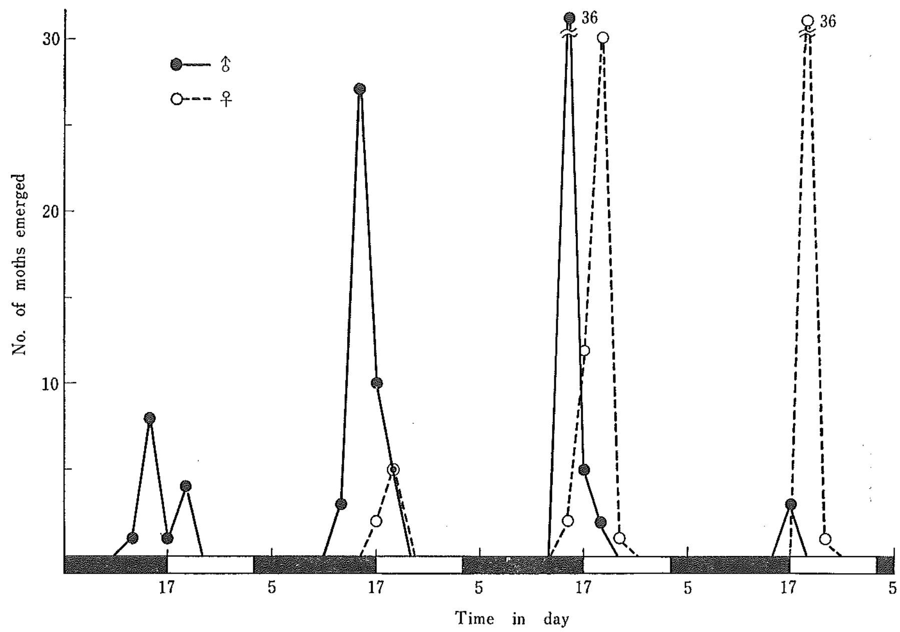

Fig. 2 Emergence feature in *Shungetsu* × *Hosho* under 10 L 14 D.

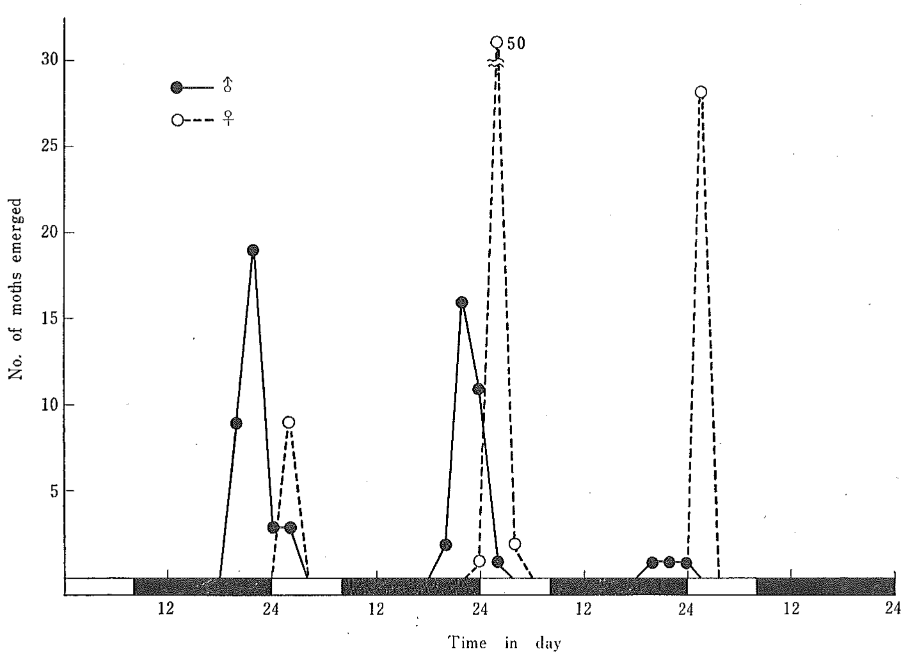

Fig. 3 Emergence feature in *Shungetsu* × *Hosho* under 8 L 16 D.

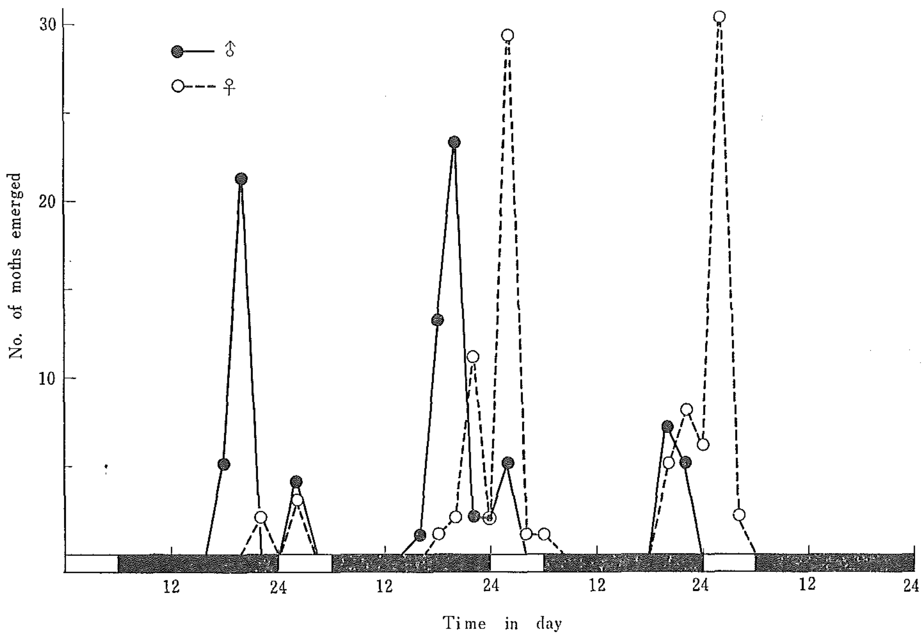

Fig. 4 Emergence feature in *Shungetsu*×*Hosho* under 6 L 18 D.

photoperiod. Most of the male and of the females emerged respectively at the late time of scotophase and the beginning time of photophase. In the third day such a tendency was exaggerated and the modal peak was divided into two onese.

8 L 16 D (Fig. 3)

The sexual difference in eclosion time became more significant than in 10 L 14 D. There appeared bimodal peaks, of which one was made by the male moths at late scotophase, and the other by the female moths at early photophase.

6 L 18 D (Fig. 4)

The appearance of emergence rhythm was closely similar to the cases of 10 L 14 D and 8 L 16 D. The male eclosion, however, showed the maximum peak at 4 hours before the end of scotophase. It was earlier 2 hours than in the above regimes. Further the minority of the female moths emerged at late scotophase.

4 L 20 D (Fig. 5)

Most of the male moths emerged at late scotophase. The modal peak, however, existed at 6 hours before the end of scotophase. In the female moths 2 modal peaks per day occurred; the one stood at two hours after photophase and the other at 2 ～ 4 hours before photophase.

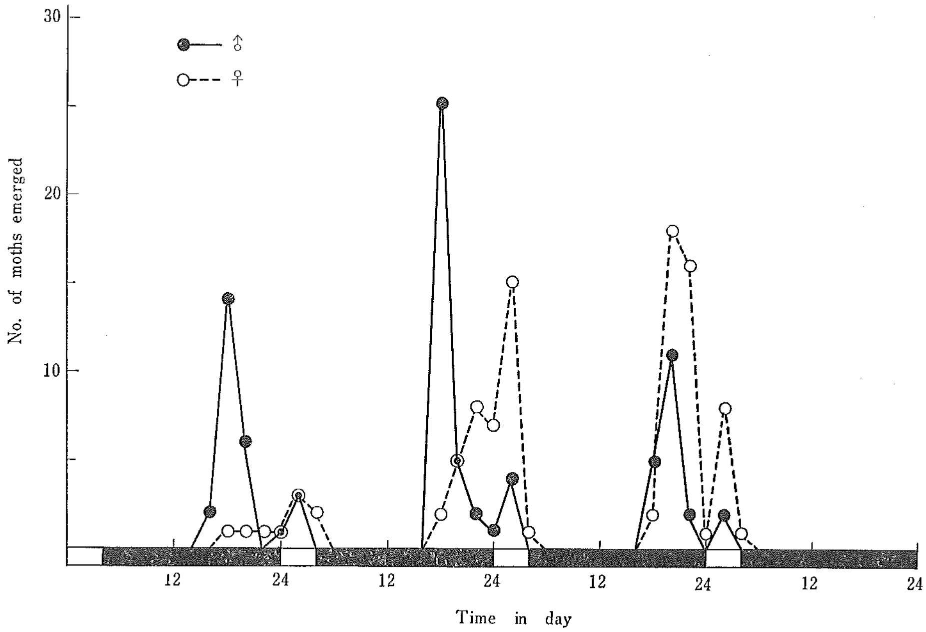

Fig. 5 Emergence feature in *Shungetsu* × *Hosho* under 4 L 20 D.

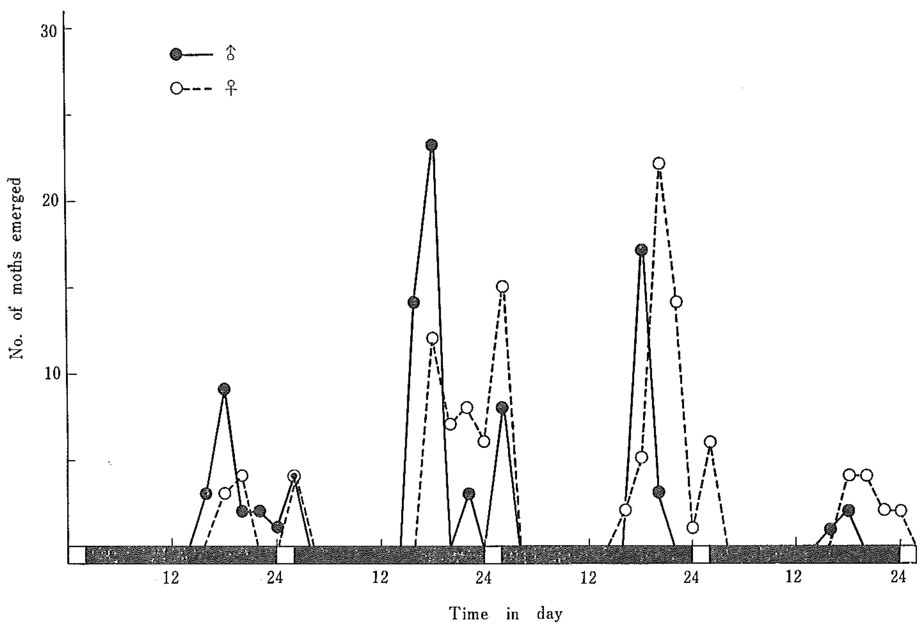

Fig. 6 Emergence feature in *Shungetsu* × *Hosho* under 2 L 22 D.

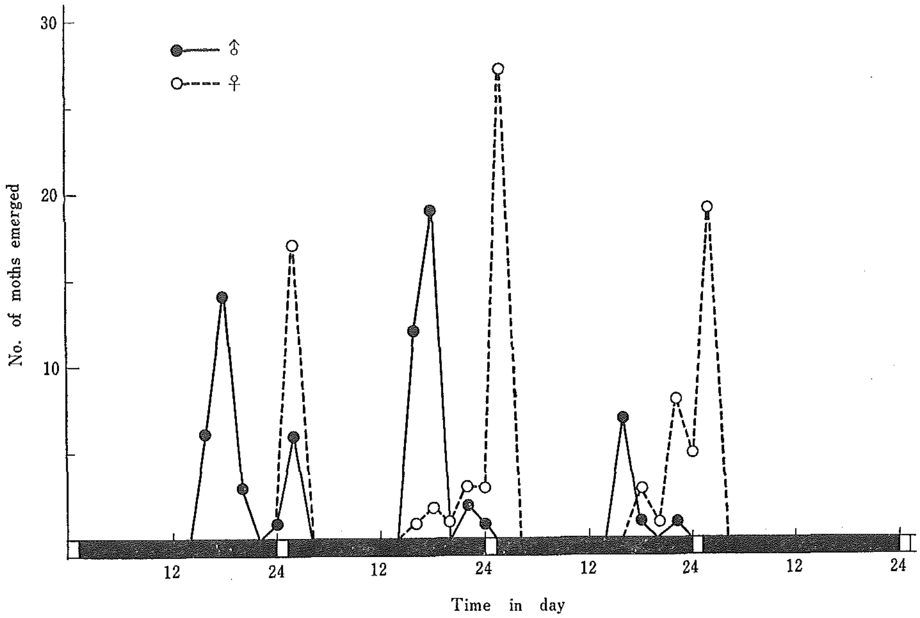

Fig. 7 Emergence feature in *Shungetsu* × *Hosho* under 1 L, 23 D.

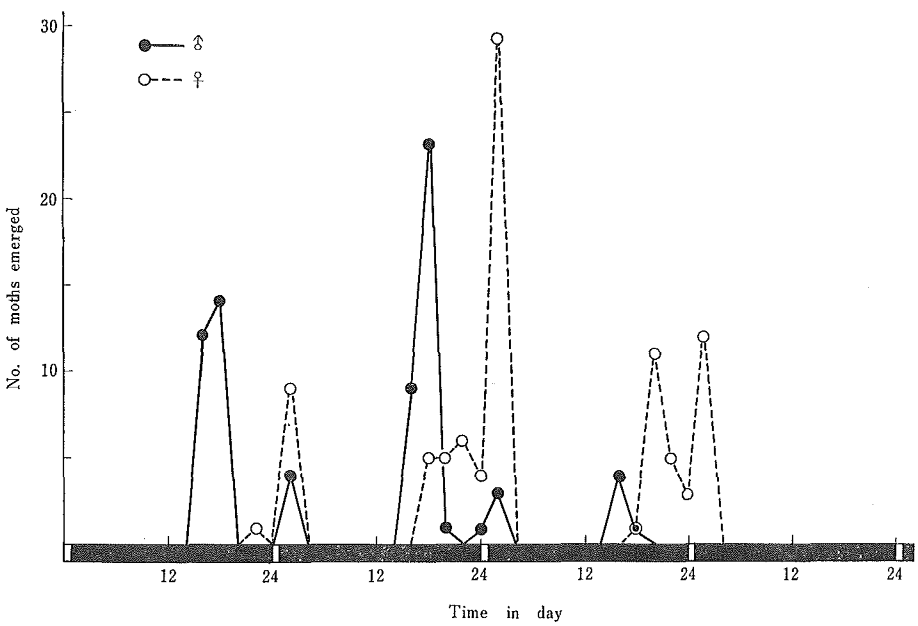

Fig. 8 Emergence feature in *Shungetsu* × *Hosho* under 30 min. -photophase.

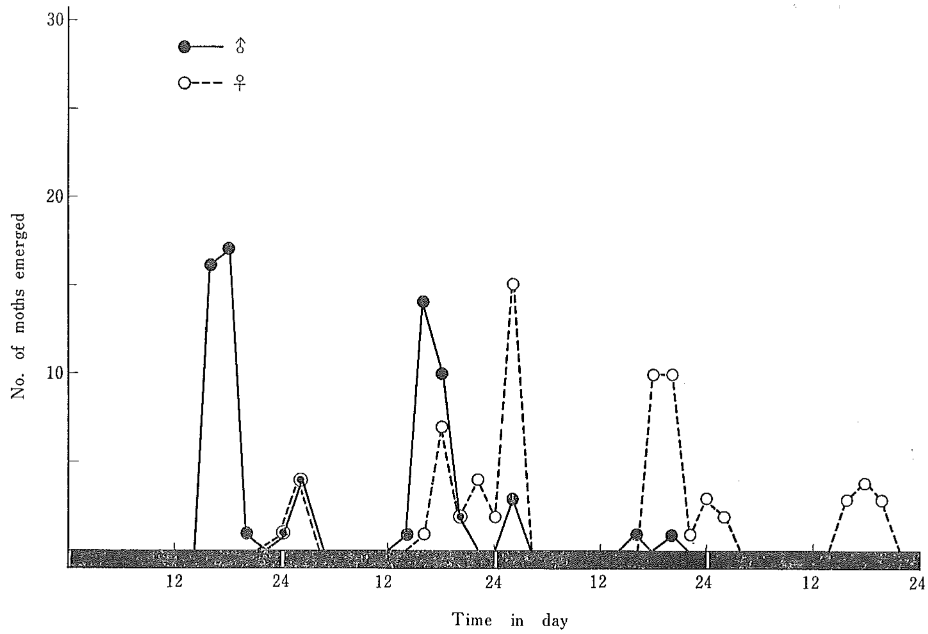

Fig. 9 Emergence feature in *Shungetsu* × *Hosko* under 15 min. -photophase.

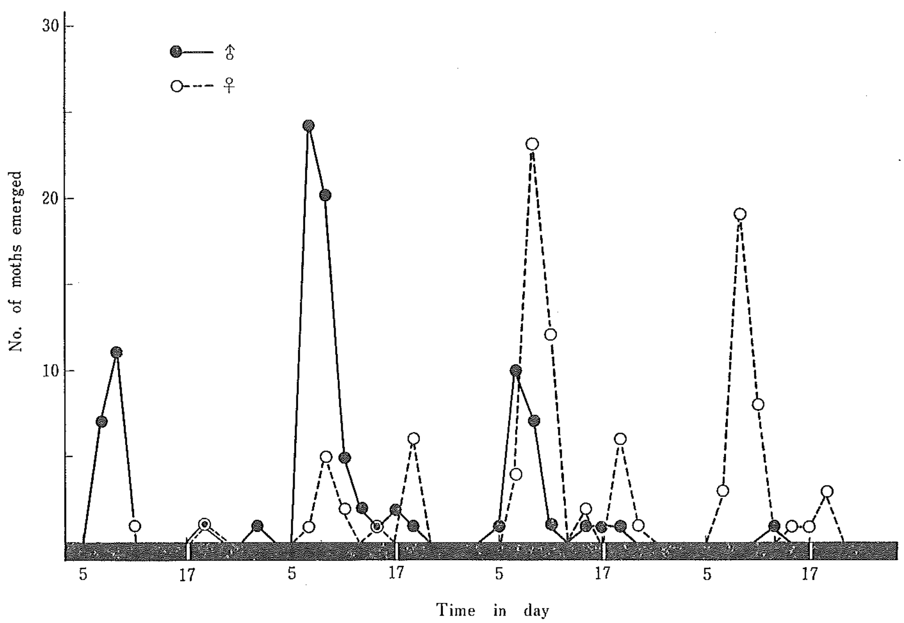

Fig. 10 Emergence feature in *Shungetsu* × *Hosho* under 5 min. -photophase.

2 L 22 D (Fig. 6)

The eclosion feature closely resembled the case of 4 L 20 D either in the male moths or in the female moths.

1 L 23 D (Fig. 7)

The difference in timing of emergence was most remarkable between the male and the female. Though some of the males eclosed at photophase and of the females at late scotophase, respectively, the modal peak was almost completely divided into two ones, which were separated 8 hours from each other.

0 : 30 L 23 : 30 D (Fig. 8)

The males and the females showed almost the same tendency in emergence feature as in 1 L 23 D. The moths seemed to be entrained by such a short exposure of illumination.

0 : 15 L 23 : 45 D (Fig. 9)

In this 15 min-illumination the emergence peak of the male moths appeared at 6 ~ 8 hours before the end of scotophase, whereas that of the female moths at 2 hours after the beginning of photophase and also at 4 ~ 6 hours before scotophase. The emerged number of the female moths became to increase at

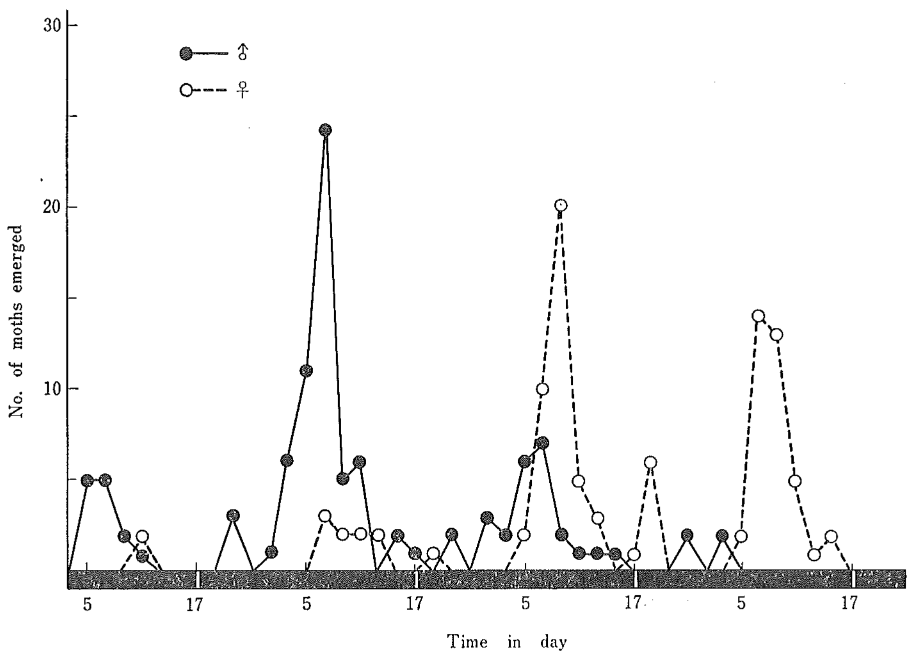

Fig. 11 Emergence feature in *Snungetsu* × *Hosho* under 1 min. -photophase.

scotophase.

0 : 05 L 23 : 55 D (Fig. 10)

When the duration of photophase was as short as 5 min., the emergence feature differed to some extent from that of the other photoperiodic regimes above-mentioned. Namely both the male and the female moths eclosed almost limitedly at 8 hours before the end of scotophase, though a small number of the female moths emerged at photophase. It is evident that the daily eclosion of the moths is entrained by such a photoperiodic stimulation.

0 : 01 L 23 : 59 D (Fig. 11)

The emergence pattern was closely similar to that of the free-running rhythm in the constant darkness (Fig. 1B). The diel rhythmicity of emergence, however, was maintained by such a short light stimulation as 1 min.

2. *Shinki* × *Ryoho*

9 L 15 D (Fig. 12)

In this regime, as above-described, the male and the female moths showed a tendency to emerge at several hours before and after onset of light, respectively. Only the second modal peak was divided into two ones.

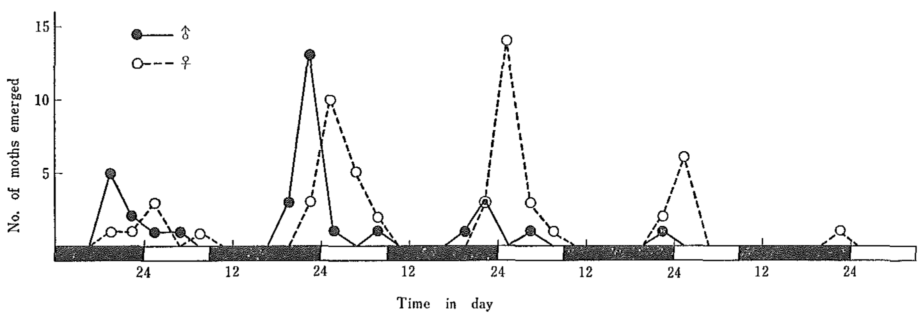

Fig. 12 Emergence feature in *Shinki* × *Ryoho* under 9 L 15 D.

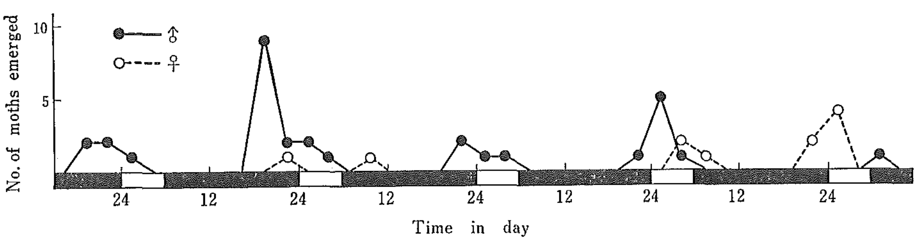

Fig. 13 Emergence feature in *Shinki* × *Ryoho* under 6 L 18 D.

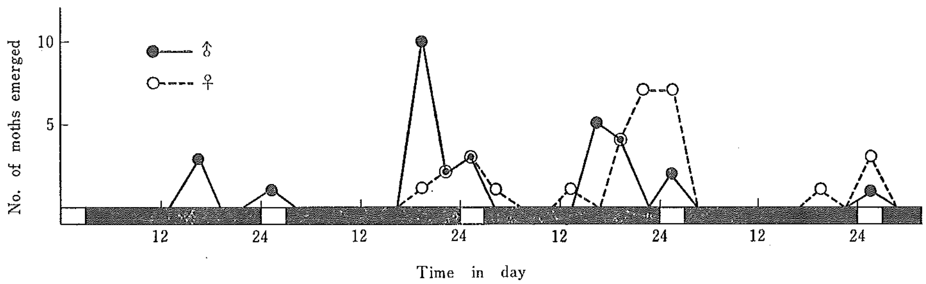

Fig. 14 Emergence feature in *Shinki* × *Ryoho* under 3 L 21 D.

6 L 18 D (Fig. 13)

The emergence rhythm was clearly entrained by the photoperiod. There occurred, however, no bimodal peak.

3 L 21 D (Fig. 14)

The tendency that the male moths emerged mainly at late scotophase and the female moths at photophase was recognized, but the bimodality of eclosion rhythm was not so distinct.

3. *Ryoho* × *Shinki*

9 L 15 D (Fig. 15)

In this case the emergence feature was hardly different from that of the above regime.

6 L 18 D (Fig. 16)

As shown in the figure only a small number of individuals eclosed in the day and the emergence rhythm was not composed.

3 L 21 D (Fig. 17)

The time lag of emergence between the male and the female was detectable

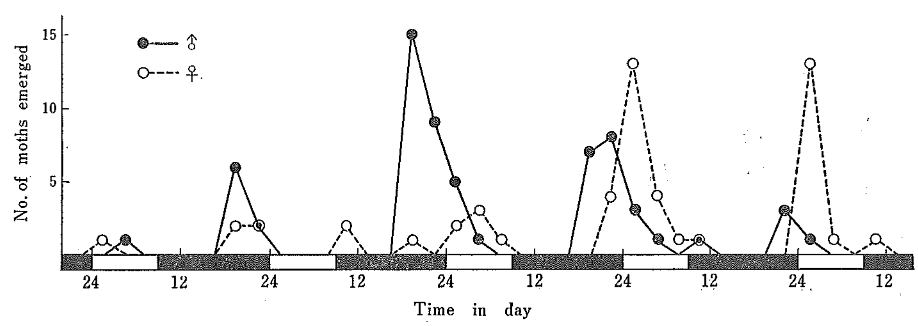

Fig. 15 Emergence feature in *Ryoho* × *Shinki* under 9 L 15 D.

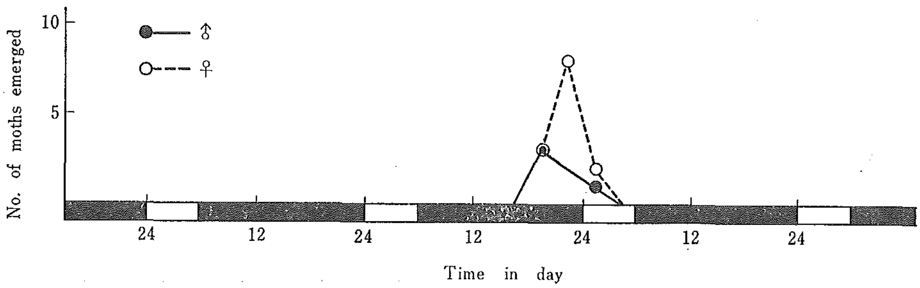

Fig. 16 Emergence feature in *Ryoho* × *Shinki* under 6 L 18 D.

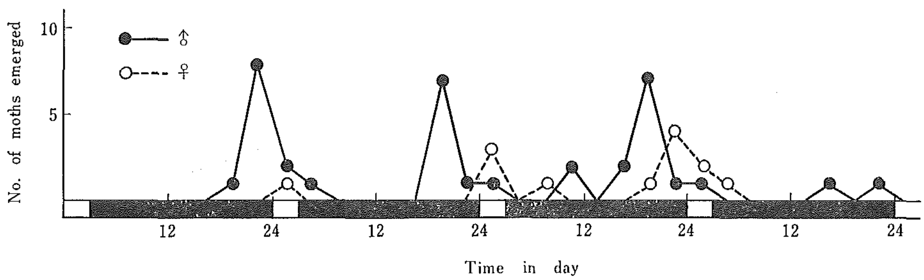

Fig. 17 Emergence feature in *Ryoho* × *Shinki* under 3 L 21 D.

to some extent. The modal peak of emergence was incompletely separated.

CONSIDERATION

Most of the Lepidopterous insect pupae emerge once a day with a daily rhythmicity. Some examples, however, are known, in which the emergence rhythm consists of two peaks per day. One example is the case of the Mediterranean flour moth, *Ephestia kühniella*. Its adult eclosion takes place twice a day, viz. at late afternoon and early evening or at late photophase and early scotophase (BREMER, 1926). The phenomenon seems to be caused by the thermal factor, too (SCOTT, 1936 : MORIARTY, 1959).

The silkworms have generally a unimodal type of rhythmic emergence, of which the peak appears from late scotophase to early photophase (KIMURA, 1952, 1953 : KOIZUMI et al., 1960). Recently KOBAYASHI and KOYAMA (1974) has pointed out the fact that the peak is sharply divided into two ones when

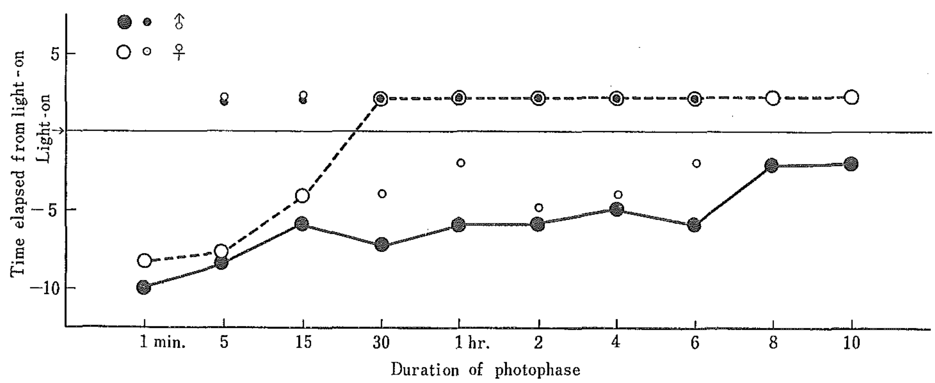

Fig. 18 Emergence time from light-on stimulation in *Shungetsu* × *Hosho.*
Large circle and small circle show each position of large peak and small peak of emergence, respectively. The same expression is presented in Fig. 19.

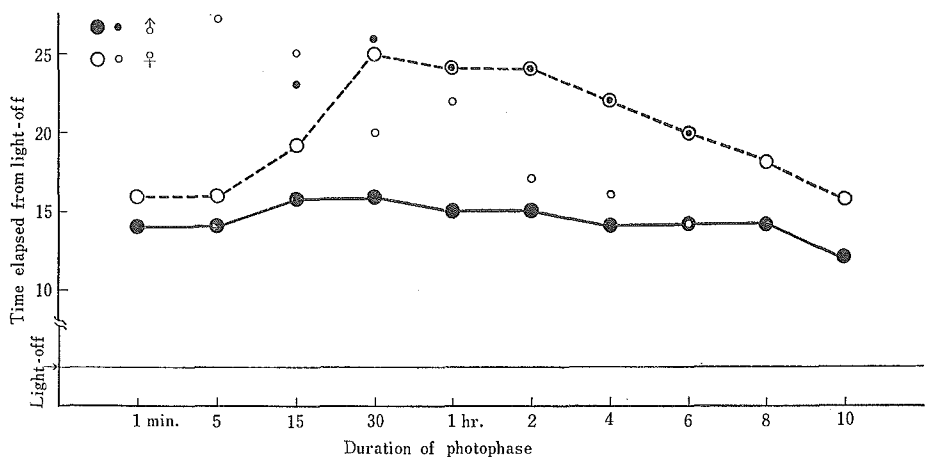

Fig. 19 Emergence time from light-off stimulation in *Shungetsu* × *Hosho.*

the pupae were exposed to the short-day photoperiod such as 3L21D, but the reason remains unsolved.

As seen in the results of this experiment, the male moths emerged so much faster than the female moths when they were confined in a constant darkness, whereas no sexual difference existed endogenously in the emergence time of day (Fig. 1B). Nevertheless when the pupae were subjected to the short-day photoperiods, photophases of which were shorter than 6 hours, the male and the female tended to emerge at late scotophase and early photophase, respectively. The tendency was exaggerated as shortening of photophase, though it disappeared under such an extreme short-day as less than 30 minutes illumination.

Fig. 18 and 19 indicate the relation between the length of photophase and the time of each emergence peak regarding the case of *Shungetsu* × *Hosho*. Observing Fig. 18 it is ascertained that the majority of the female moths emerged limitedly at 2 hours after light-on time in the regimes of more than 30 min. -illumination. Taking the fact into consideration the emergence of the female moths seems to be arisen mainly by the light stimulation. On the other hand, the majority of the male moths eclosed at several hours before light-on time. The time lag of the emergence peak between sexes was not detectable under the regimes of less than 15 min. -illumination, which quite significant within 30 min. -illumination to 6 hours-one. Namely the separation of the modal peak occurs in the latter regimes. In Fig. 19 the majority of the male moths emerged almost constantly at 12 ~ 16 hours after light-off time throughout all the regimes, while the emergence time of the female moths varied suggesting the light-off stimulation is not the causal factor of emergence. In the fall web-worm, *Hyphantria cunea* the light-off or the temperature drop is a time cue for the adult emergence (HIRAI, 1969, 1972).

In the silkworm, however, it may be difficult to consider that the male emergence is directly caused by the light-off stimulation, because the duration between the light-off and emergence times is very long for the photic response (Fig. 19), some males usually emerge when exposed to light and some females eclose at late scotophase (Fig. 18), and the sexual difference would not be so evident in photic reaction. EDWARDS (1962) revealed that sexual difference existed in the circadian rhythm of activities of some nocturnal moths, but the causal factor may be different considerably from the case of the silkworm. At present the authors have the opinion that the male pupae are more steadily entrained by the photic conditions than the female pupae. The tree reason remains unsolved, however.

Anyway it becomes clear that the bimodality of emergence rhythm in the silkworm moths is caused by the time lag of emergence between sexes. But it seems to differ according to strain or diet as seen in *Shinki* × *Ryoho* and *Ryoho* × *Shinki* (Fig. 12 ~ 17).

PITTENDRIGH and BRUCE (1959) showed that such an extremely short flash as 1/2,000 sec. would phase set the emergence rhythm of dark-reared *Drosophila* cultures. At least 1 min. -illumination may be a phase-setting stimulus in the silkworm. Further it is suggested that there is a fair possibility for separation of the sexes by making use of photoperiodic regime in practical sericulture.

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