Effect of photoperiod on the host attachment and development in the tick, *Haemaphysalis longicornis*

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Abstract: The effects of photoperiod on host attachment activity and development in the tick, *Haemaphysalis longicornis*, were observed at 25°C under two photoperiodic conditions (10L-14D and 16L-8D). The development of engorged larvae and engorged nymphs was hardly affected by photoperiod during the stages from egg to engorged larva or engorged nymph. However, the host attachment activities of unfed nymphs and unfed adults were significantly suppressed by exposure to a short-day photoperiod (10L-14D) during the stages from egg to unfed nymph or unfed adult. These results suggest that diapause occurs in unfed nymphs and unfed adults which were exposed to short-day photoperiod, but not in engorged larvae and engorged nymphs. In Saitama Prefecture, the disappearance of *H. longicornis* nymphs in autumn may be related to the diapause of the unfed nymphs. However, the low activity of the adults in autumn can not be fully explained by the diapause, because the adults develop in the long-days of spring to summer.

INTRODUCTION

Haemaphysalis longicornis Neumann is well known as the most harmful tick in pastures (Kitaoka *et al.*, 1975; Ootake *et al.*, 1985) and as a vector for theileriosis caused by *Theileria sergenti* Yakimoff and Dekhtereff (Ishii and Ishihara, 1951). Therefore, there have been many ecological studies on this tick in Japan (Namba, 1958; Yoshida, 1975, 1980; Chikaki, 1976; Shiraishi *et al.*, 1982, 1989; Ito *et al.*, 1983; Kakuda *et al.*, 1990). However, the life cycle of this tick

is not fully analyzed in these reports. In general, the life cycles of ticks are controlled by various meteorological factors. Especially, diapause controlled by photoperiod has an important role in the seasonal regulation of the life cycles of many tick species (Belozarov, 1982). The present study examines the effect of photoperiod on host attachment activity and development in the tick, *H. longicornis*, and clarifies whether diapause occurs in this tick or not.

MATERIALS AND METHODS

Tick rearing. *H. longicornis* used in this study were derived from adults collected by the flagging method at a hilly area in Saitama

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Prefecture. The adults collected were fed on the ears of domestic rabbits in the laboratory, maintained at $25\pm 3^{\circ}\text{C}$, and with a 16L-8D photoperiod (16 hr light/8 hr dark). Ear bags were used for feeding adult ticks on rabbits. The engorged females dropped off from the host rabbits were placed in tick containers (Petri dishes 4.5 cm in diameter) with wet filter papers at the bottom, and kept in an incubator (25°C , 16L-8D photoperiod). The eggs deposited by the females were placed daily in tick containers (3.0 cm in diameter) and incubated at 25°C under two photoperiodic conditions (10L-14D and 16L-8D). Two groups (10L-14D and 16L-8D groups) of the larvae and nymphs used in this study were derived from these eggs reared under each photoperiodic condition. Two groups of the larvae and nymphs were fed on the ears of domestic rabbits under $25\pm 3^{\circ}\text{C}$ and each photoperiodic condition. Two groups of the engorged larvae and engorged nymphs detached were placed in tick containers, and reared at 25°C under each photoperiodic condition.

Effect of photoperiod on the host attachment of unfed ticks. The "ear bag method" was used in this experiment. Each of the two groups of unfed nymphs and unfed adults (1 to 4 months old after molting) was taken at an infestation density from 60 to 100 per ear bag for nymphs, and from 15 to 20 per ear bag for adults. The ear bags which covered the ears of a domestic rabbit were fixed with adhesive plaster on the ear base. The tick-infested rabbits were kept in the laboratory, maintained at $25\pm 3^{\circ}\text{C}$, and with a 16L-8D photoperiod. The attached ear bags were kept for 8 hr after infestation and subsequently removed from the ears of domestic rabbits. The number of attached ticks was counted for each group to calculate the rate of host attachment.

Effect of photoperiod on the development of engorged ticks. Each of two groups of detached engorged larvae and engorged nymphs from the host rabbits on the same day were placed together in batches of 10 to 15 individuals in tick containers for larvae, and one to 5 individuals for nymphs, and

kept in incubators at 25°C under each photoperiodic condition. The developmental periods (days from drop-off to the following ecdysis) and the percentages of molting in engorged larvae and engorged nymphs were recorded at each photoperiodic condition.

RESULTS

Effect of photoperiod on the host attachment of unfed ticks

As shown in Table 1, the mean rates of host attachment in nymphs exposed to 16L-8D and 10L-14D photoperiods during the stages from egg to unfed nymph were 60.4 and 34.2%, respectively. There was a significant difference in the rate of host attachment in nymphs between 16L-8D and 10L-14D ($\chi^2=56.21$, $p<0.001$). On the other hand, the rate of host attachment of adults exposed to 16L-8D during the stages from egg to unfed adult averaged 74.5%, but that of adults exposed to 10L-14D was 0% (Table 2). A significant difference was found between the rates of host attachment in adults exposed to 16L-8D and in those exposed to 10L-14D ($\chi^2=83.71$, $p<0.001$).

Table 1 Percentage of host attachment of *H. longicornis* nymphs exposed to two photoperiodic conditions from egg to unfed nymph.

Photoperiodic conditions (hr)	No. of rabbits used	Total No. of nymphs used	Mean % attachment
10L-14D	4	386	34.2
16L-8D	4	432	60.4

Table 2 Percentage of host attachment of *H. longicornis* adults exposed to two photoperiodic conditions from egg to unfed adult.

Photoperiodic conditions (hr)	No. of rabbits used	Total No. of adults used	Mean % attachment
10L-14D	2	78	0
16L-8D	2	55	74.5

Table 3 Development of engorged *H. longicornis* larvae reared at 25°C under two photoperiodic conditions from egg to engorged larva.

Photoperiodic conditions (hr)	No. of engorged larvae examined	Mean developmental period in days* ($\pm$ S.D.)	% molting
10L-14D	110	12.2 ($\pm$ 0.9)	100
16L-8D	123	12.8 ($\pm$ 1.6)	96.7

* Days from drop-off to the following ecdysis. S.D.: standard deviation.

Table 4 Development of engorged *H. longicornis* nymphs reared at 25°C under two photoperiodic conditions from egg to engorged nymph.

Photoperiodic conditions (hr)	No. of engorged nymphs examined	Mean developmental period in days* ($\pm$ S.D.)	% molting
10L-14D	86	12.7 ($\pm$ 1.0)	97.7
16L-8D	47	14.3 ($\pm$ 1.0)	100

* Days from drop-off to the following ecdysis. S.D.: standard deviation.

Effect of photoperiod on the development of engorged ticks

As shown in Table 3, the mean developmental periods of engorged larvae exposed to 16L-8D and 10L-14D photoperiods during the stages from egg to engorged larva were 12.8 and 12.2 days, respectively. The molting percentages of the engorged larvae exposed to 16L-8D and 10L-14D were 96.7 and 100%, respectively. On the other hand, the mean developmental periods of engorged nymphs exposed to 16L-8D and 10L-14D photoperiods during the stages from egg to engorged nymph were 14.3 and 12.7 days, respectively (Table 4). The molting percentages of the engorged nymphs exposed to 16L-8D and 10L-14D were 100 and 97.7%, respectively.

DISCUSSION

Diapause in ticks is classified into two types; *i.e.*, behavioral (host-seeking) diapause and morphogenetic (developmental) diapause (Belozero, 1982). Behavioral diapause is characterized by the suppression of host attachment activity (host-seeking activity) in unfed ticks, and morphogenetic diapause results from the delay in reproduction

and development in engorged ticks. In this study, the development of engorged larvae was little affected by the photoperiodic conditions that they experienced during the stages from egg to engorged larva. Further, photoperiod had little effect on the development of engorged nymphs, although the developmental period was slightly prolonged only when they were exposed to a long-day photoperiod (16L-8D) during the stages from egg to engorged nymph. These results suggest that morphogenetic diapause does not occur in engorged larvae and engorged nymphs. On the other hand, the host attachment activities of unfed nymphs and unfed adults were significantly suppressed when they were exposed to a short-day photoperiod (10L-14D) during the stages from egg to unfed nymph or adult. This suggests that behavioral diapause occurs in unfed nymphs and unfed adults as a result of exposure to short-day photoperiod.

At a hilly area in Saitama Prefecture, *H. longicornis* nymphs were collected on vegetation from April through July with a peak in May, and the adults from May through September with a peak in July (Fujimoto, unpublished). The seasonal activities of the nymphs and adults on vegetation were characterized by the disappearance or low activity

in autumn. The diapause in unfed *H. longicornis* nymphs induced by short-days photoperiod may be related to the disappearance of the nymphs in autumn in Saitama Prefecture, because most of the nymphs develop in the short-days of autumn. However, the low activity of *H. longicornis* adults in autumn can not be fully explained by the diapause, because the adults develop in the long-days of spring to summer.

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摘 要

フタトゲチマダニの宿主への寄生と発育に及ぼす日長の影響

フタトゲチマダニの幼虫, 若虫, 成虫の宿主への寄生と発育を 25°C 一定で, 日長条件 (10L-14D, 16L-8D) を変えて観察した。飽血幼虫および飽血若虫の発育は, 卵期から長日条件 (16L-8D) で飼育しても, 短日条件 (10L-14D) で飼育しても, ほとんど変わらなかった。しかし, 未吸血若虫および未吸血成虫の宿主への寄生は卵期から短日条件で飼育した場合, 著しく阻害された。これらの結果は, フタトゲチマダニの未吸血若虫と未吸血成虫は, 短日条件で育った場合, 休眠に入るが, 飽血幼虫と飽血若虫は, 日長条件にかかわらず, 休眠に入らないことを示唆する。

埼玉県において, 秋に若虫が出現しないのは, 秋の短日条件下で発育した若虫が休眠に入るためかもしれない。しかし, 秋における成虫の不活発な活動は, 成虫が春から夏の長日条件下で発育することから, 休眠によって説明できない。