Research Note

An experimental analysis on the low activity of *Amblyomma testudinarium* Koch nymphs (Acari: Ixodidae) in late autumn, with special reference to behavioral diapause

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Abstract: The feeding activity of *Amblyomma testudinarium* Koch nymphs that were placed outdoors from engorged larvae was observed under laboratory conditions (24°C, 10L–14D photoperiod) from October to December 2001. The rates of engorgement of the nymphs ranged from 48.3 to 55.0% in October to December. The rate of engorgement was not reduced even in December. These results suggest that *A. testudinarium* nymphs do not enter behavioral diapause on exposure to the short days of autumn. Since the vertical activity of the nymphs in the plastic cylinders becomes lower as the temperatures of autumn drop, their low activity in late autumn appears to be a simple quiescence due to the temperature.

Key words: *Amblyomma testudinarium*, nymphal tick, late autumn, low activity, behavioral diapause

INTRODUCTION

The tick, *Amblyomma testudinarium* Koch, which is known to be a reserve host of the spotted fever group rickettsiae (Fujita et al., 1996), is widely distributed in the south-western part of Japan (Takada, 1990). The nymphs of this tick were collected from floor vegetation from spring to autumn in the Rokko Mountains of Hyogo Prefecture (Fujimoto, 2000). However, the activity of the nymphs on floor vegetation was abruptly reduced in late autumn (November) (Fujimoto unpublished). It is generally known that ixodid nymphs enter behavioral diapause as a result of exposure to the short days of autumn (Fujimoto, 1993, 1994, 2000). Thus, the low activity of the nymphs in late autumn may be also caused by behav-

ioral diapause. On the other hand, Kitaoka and Suzuki (1974) reported that *A. testudinarium* nymphs were found on floor vegetation even in December and January on Amami-Oshima Island. This may suggest that the nymphs do not exhibit behavioral diapause in late autumn. The objective of this study is to clarify whether or not *A. testudinarium* nymphs enter behavioral diapause on exposure to the short days of autumn.

MATERIALS AND METHODS

Ticks: Larval ticks were collected by the flagging method in the Rokko Mountains of Hyogo Prefecture, in May or June 2001. The larvae fed on the ears of domestic rabbits that were maintained in a laboratory at 24°C under a 16L–8D photoperiod (16 h light/8 h dark). The detached

engorged larvae were kept in Petri dishes and placed outdoors (Saitama Medical School campus) where they were not directly exposed to sunlight or rain. The nymphs that molted from these engorged larvae (molted in July to September 2001) were used in this study.

Nymphal activity in plastic cylinders: The vertical activity of the nymphs in plastic cylinders (Fujimoto, 1995) was observed in order to clarify their activity in late autumn. Observations were made in a 3-month period from October to December 2001 using the same methods as in the previous report (Fujimoto, 1995). Two plastic cylinders were used in this study. Thirteen or fourteen nymphs were released on bog moss in each plastic cylinder. The plastic cylinders were placed outdoors where the nymphs were not directly exposed to sunlight or rain. To keep the bog moss in the plastic cylinders wet, water was added to the black soil in the flower pot at appropriate intervals. The nymphs which were attached to the nylon cloth or internal walls of the plastic

cylinders were counted daily as active ticks, and their percentage was then calculated.

Host-feeding activity: The host-feeding activity was observed on the ears of domestic rabbits from October to December 2001. The nymphs were allowed to feed in the ear bag (30 nymphs per bag) that covered the ears of domestic rabbits under laboratory conditions (24°C, 10L–14D photoperiod) for eight hours after infestation. The number of engorged nymphs was counted in order to calculate the engorgement rate. The host rabbits were used only once.

RESULTS AND DISCUSSION

Nymphal activity in plastic cylinders

Figure 1 shows the vertical activity of *A. testudinarium* nymphs (which were developed in the summer) in the plastic cylinders during the period from October to December 2001. The nymphs formed a peak early in October. Thereafter, however, the activity gradually decreased and

Fig. 1. Monthly change in the vertical activity of *A. testudinarium* nymphs in the plastic cylinders. The percentages of active ticks are indicated as the means of the tick numbers observed from 3 to 5 days.

Temperatures are indicated as the means of the temperatures of the time when observations were conducted.

Table 1. Monthly change in the engorgement rates of *A. testudinarium* nymphs that were placed outdoors from the engorged larvae.

Month	No. of rabbits used	Total no. of nymphs used	No. of nymphs engorged	Mean % engorgement
October	2	60	29	48.3
November	2	60	33	55.0
December	1	60	31	51.7

was barely observed in late December.

Host-feeding activity

Table 1 shows the monthly change in the rates of engorgement of *A. testudinarium* nymphs. The rate of engorgement of *A. testudinarium* nymphs that were placed outdoors from the engorged larval stage was 48.3% when they attached to hosts in October 2001. However, the rate of engorgement did not decrease in late autumn. The rate was 51.7% even in December. These rates of engorgement were similar to that (52.5%; $n=120$) of the field-caught nymphs in May 2002 (Fujimoto unpublished). Thus, the host-feeding activity of *A. testudinarium* nymphs that have been placed outdoors does not appear to be suppressed by the exposure to the short days of autumn; i.e., they do not enter behavioral diapause in late autumn. The vertical activity of the nymphs in the plastic cylinders became lower as the temperatures of autumn dropped (Fig. 1). Thus, their low activity in late autumn appears to be a simple quiescence caused by the temperature. *A. testudinarium* is known to be a tropical tick which is widely distributed in Southeast Asia (Yamaguti et al., 1971). Thus, the temperatures of late autumn may be a little too low for the activity of the nymphs. The activity of *A. testudinarium* nymphs in winter on Amami-Oshima Island (Kitaoka and Suzuki, 1974) appears to be due to the warm climate.

The diapausing ticks have the advantage of being more resistant to unfavorable temperatures (Belozеров, 1982). There-

fore, the existence of diapause in ticks appears to have an effect on their distribution. *A. testudinarium* is widely distributed in the south-western part of Japan, but not in the northern part (Takada, 1990). One reason why the distribution of *A. testudinarium* is limited in Japan may be due to the non-diapause of the nymphs. However, *A. testudinarium* nymphs did not enter behavioral diapause but exhibited morphogenetic diapause (Fujimoto, 2001). Therefore, further studies on the existence of diapause of the other developmental stages (larva and adult) will be necessary to analyze the limited distribution of *A. testudinarium*.

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