[SHORT COMMUNICATION]

A Preliminary Study on the Drop-off Rhythms of the Engorged Larvae and Nymphs of *Ixodes ovatus* Neumann (Acari: Ixodidae)

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INTRODUCTION

The timing of drop-off from their hosts is important in order for ticks to synchronize with host behavior patterns (Belozerov, 1982; Sonenshine, 1993). Therefore, there have been many studies on the daily drop-off rhythms of engorged ticks (Amin, 1970; Arthur, 1962; Camin, 1963; Doube, 1975 a, b; George, 1971; Hadani and Rechav, 1969, 1970; Hadani and Ziv, 1974; Kitaoka, 1962; Wharton and Utech, 1970). In Japan, however, there is no data about the daily drop-off rhythms of engorged ticks except for that of engorged *H. longicornis* females identified as *H. bispinosa* (Kitaoka 1962).

The tick, *Ixodes ovatus* Neumann, which is a known vector of *Borrelia* spirochete (Miyamoto et al., 1992), is widely distributed in Japan. The adults of this tick are commonly captured by the flagging method, but the larvae and nymphs are not (Fujimoto et al., 1987). This appears to be related to the larval and nymphal habit of attaching to hosts (wild mice and moles) in their burrows (Asanuma, 1965). Belozerov (1982) theorized that, in nest-dwelling ticks, the drop-off rhythm is either nocturnal, when hosts are active during the day and shelter in nests at night, or diurnal, when hosts shelter during the day and are active at twilight or during the night. The hosts (wild mice and moles) of *I. ovatus* larvae and nymphs are generally nocturnal types; thus, the drop-off rhythms of their larvae and nymphs appear to be diurnal. However, the adults change the hosts from small-sized mammals into medium- or large-sized mammals (Asanuma, 1965). Therefore, the nymphs, which are the preceding instar for adults, may need to have a nocturnal drop-off rhythm because adults cannot find the new hosts in the diurnal drop-off rhythm. The purpose of this study is to clarify whether the daily drop-off rhythms of engorged *I. ovatus* larvae and nymphs are diurnal.

MATERIALS AND METHODS

Tick rearing. Adult ticks were collected in the Chichibu Mountains of Saitama Prefecture by the flagging method, and were fed on the ears of domestic rabbits that were maintained in the laboratory at $25 \pm 3^\circ\text{C}$ in a 14L-10D photoperiod (14 hr light / 10 hr dark). The detached engorged females were placed in Petri dishes (3 or 4.5 cm in diameter) with a sheet of wet filter paper, and were kept in the same laboratory. The eggs deposited by these females were collected daily in Petri dishes and were kept in the laboratory. The larvae used in this study were derived from these eggs. The nymphs used in this study were derived from the engorged larvae that were fed on mice in the laboratory.

Tick feeding experiment. Unfed ticks, two to five weeks old after hatching or molting, were placed on the head of a mouse with an infestation density of 100 per mouse for larvae, and 30 for nymphs, and were kept in a laboratory that was maintained at $25 \pm 3^\circ\text{C}$ in a 14L-10D photoperiod. The feeding experiments on unfed ticks began at 10:00. The mice were kept in the cages for 8 hrs after infestation and were then set free, and were transferred to laboratories that were maintained at $25 \pm 3^\circ\text{C}$ under the following 3 conditions: a 14L-10D photoperiod, constant darkness, and constant light. Four to six mice were used for each experimental condition. The infested mice were fed once a day at midnight. The number of the detached engorged ticks for each condition was counted at 5- or 7- hr intervals at 5, 12, 19, and 24:00, in order to calculate the drop-off rate. Observations were continued until they had all dropped.

RESULTS

As shown in Fig. 1, the engorged *I. ovatus* larvae had a diurnal drop-off rhythm, although the rhythm is not completely clear. More than 70% of the engorged larvae dropped between 5:00 and 19:00 at a 14L-10D photoperiod (Fig. 1 A). This drop-off rhythm was maintained in constant darkness and in constant light, although the engorged larvae had a relatively higher percentage of drop-off between 19:00 and 5:00 in constant light (Fig. 1 B, C).

On the other hand, the drop-off rhythm of the engorged nymphs also exhibited a similar pattern to that of the engorged larvae (Fig. 2). More than 70% of the engorged nymphs dropped between 5:00 and 19:00 in a 14L-10D photoperiod (Fig. 2 D). This dropping rhythm was unchanged in constant darkness and light (Fig. 2 E, F).

DISCUSSION

In this study, engorged *I. ovatus* larvae and nymphs exhibited a diurnal drop-off rhythm, although the rhythm is not completely clear. It is known that diurnal rhythmicity in the dropping of engorged ticks occurs in a number of ixodid species, and that the primary factor controlling the drop-off rhythm is the photoperiod, *i. e.* daily changes in the length of phases of light and darkness (Arthur, 1962; Kitaoka, 1962; Camin, 1963; Hadani and Rechav, 1969, 1970; Amin, 1970; George, 1971; Hadani and Ziv, 1974; Rechav, 1978). According to Belozarov (1982), the daily drop-off rhythms of engorged ticks have an endogenous circadian basis, which is mainly entrained by photoperiodic signals. On the other hand, the

Fig. 1. Drop-off rhythms of engorged *I. ovatus* larvae from albino mice; in a 14L-10D photoperiod (A), in constant darkness (B), and in constant light (C).

daily rhythms are also affected by certain external physical conditions, their physiological state, and movement of the infested hosts (Kitaoka, 1962; Balashov, 1972). Amin (1970) speculated that the drop-off rhythm of engorged ticks was related to circadian changes in the corticosterone levels of the hosts' blood. In this study, there is no data on the movement and physiology of the host mice. However, the diurnal drop-off rhythms of engorged *I. ovatus* larvae and nymphs appear to be mainly controlled by an endogenous circadian mechanism of regulation which is entrained by the photoperiod, because the diurnal drop-off rhythms

Fig. 2. Drop-off rhythms of engorged *I. ovatus* nymphs from albino mice; in a 14L-10D photoperiod (D), in constant darkness (E), and in constant light (F).

were maintained in constant darkness and light. The reasons why the diurnal drop-off rhythms were not completely clear are probably due to the movement or the physiological changes of the host mice.

Belozerov (1982) hypothesized that, in nest-dwelling ticks, the drop-off rhythm is diurnal when hosts shelter during the day and are active at twilight or during the night. *I. ovatus* larvae and nymphs are nest-dwelling ticks which have a habit of attaching to hosts (wild mice and moles) in their burrows (Asanuma, 1965). Furthermore, the hosts are generally nocturnal; thus, the results of this study agree with Belozerov (1982). The diurnal drop-off rhythm of the engorged larvae may make it easy for the subsequent instar (nymphs) to find the hosts because they had dropped in the hosts' burrows. However, the diurnal drop-off rhythm of the engorged nymphs is not adaptive because the subsequent instar (adults) changes the host from small-sized into large- or medium-sized mammals (Asanuma, 1965). Fujimoto (1997) found that *I. ovatus* females entered behavioural diapause and postponed host-feeding and -seeking activities during a period of about 10 months after molting occurred. One of the reasons why the engorged nymphs dropped in the hosts' burrows by

the diurnal drop-off rhythm may be due to the need to protect the subsequent instar (adults) from the cold and dryness of winter.

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