Morphogenetic diapause of engorged *Amblyomma testudinarium* Koch nymphs (Acari: Ixodidae) induced by short-day photoperiods

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**Abstract:** In order to clarify whether or not engorged *Amblyomma testudinarium* Koch nymphs exhibit morphogenetic diapause, their development was observed at 25°C under four photoperiodic conditions (10L–14D, 11L–13D, 12L–12D, and 16L–8D). The molting of the engorged nymphs was little affected by the exposure to 12L–12D and 16L–8D photoperiods. However, the molting was retarded or arrested by the exposure to 10L–14D and 11L–13D photoperiods. These results suggest that engorged *A. testudinarium* nymphs enter morphogenetic diapause under short-day photoperiods. Judging from the percentage of molting, their critical photoperiod for the induction of diapause appeared to be close to 11 hr. Thus, a part of the *A. testudinarium* nymphs that are active in autumn enter morphogenetic diapause and hibernate in the engorged state.

**INTRODUCTION**

Morphogenetic diapause, which results from a block of some essential steps in the development and reproduction, is found in many engorged ixodid ticks (Belozеров, 1982). However, the occurrence of morphogenetic diapause is limited to ixodid ticks which are distributed in the Palearctic region (Belozеров, 1982).

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. The nymphs of this tick were found on vegetation in autumn in the Rokko Mountains of Hyogo Prefecture (Fujimoto, 2000). Fujimoto (1993, 1994a, b, 1998) reported that morphogenetic diapause occurred in engorged *Ixodes* and *Haemaphysalis* nymphs under 12L–12D and 10L–14D photoperiods. This suggests that the engorged *Ixodes* and *Haemaphysalis* nymphs enter morphogenetic diapause under the short days of autumn. However, *A. testudinarium* nymphs may not exhibit morphogenetic diapause in the short days of autumn, because they are widely distributed in the tropics (the Oriental region) where the change in day length is minimal throughout the year (Yamaguti et al., 1971). The objective of this study is to clarify whether or not *A. testudinarium* nymphs enter morphogenetic diapause under short-day photoperiods.

**MATERIALS AND METHODS**

Ticks. *A. testudinarium* larvae were collected by the flagging method in the...
Rokko Mountains of Hyogo Prefecture in May, 1999. The larvae were fed on the ears of domestic rabbits that were maintained in a laboratory at 23°C under a 16L–8D (16 hr light/8 hr dark) photoperiod. The detached larvae were placed in Petri dishes (3 or 4.5 cm in diameter) with wet filter paper on the bottom, and kept in the same laboratory as above. The nymphs that molted from these larvae were used in this study.

Effect of photoperiod on nymphal development. The nymphs (7-to 8-month-old) fed on the ears (infestation density of 20 to 50 per ear) of the domestic rabbits maintained in the laboratories at 23°C under four photoperiodic conditions (10L–14D, 11L–13D, 12L–12D, and 16L–8D). The detached engorged nymphs were placed in the Petri dishes, and kept in incubators at 25°C under the same photoperiodic conditions. The developmental period (days from drop-off to the following ecdysis) and molting percentage were recorded for each photoperiodic condition. Observations ended 60 days after detachment.

RESULTS AND DISCUSSION

Table 1 shows the development of engorged A. testudinarium nymphs reared at 25°C under four photoperiodic conditions.

Table 1. Development of engorged A. testudinarium nymphs reared at 25°C under four photoperiods from the host feeding.

<table>
<thead>
<tr>
<th>Photoperiods from the host feeding (hr)</th>
<th>No. of engorged nymphs examined</th>
<th>*% molting</th>
<th>**Mean developmental period in days</th>
</tr>
</thead>
<tbody>
<tr>
<td>10L–14D</td>
<td>34</td>
<td>0</td>
<td>60.0&lt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>28.5 (n = 6)</td>
</tr>
<tr>
<td>11L–13D</td>
<td>9</td>
<td>66.7</td>
<td>60.0&lt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>27.9 (n = 3)</td>
</tr>
<tr>
<td>12L–12D</td>
<td>16</td>
<td>93.8</td>
<td>60.0&lt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>29.4 (n = 15)</td>
</tr>
<tr>
<td>16L–8D</td>
<td>28</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

* Observations ended 60 days after detachment.

** Days from drop-off to the adult ecdysis.

The percentage of molting in the engorged nymphs was very high (93.8–100%) when they had been exposed to 16L–8D or 12L–12D photoperiods. The mean developmental periods of the engorged nymphs at 25°C under 16L–18D and 12L–12D photoperiods were 29.4 and 27.9 days, respectively. On the other hand, the development of the engorged nymphs was retarded or arrested when they were exposed to 11L–13D and 10L–14D photoperiods. 33.3% of the engorged nymphs that had been exposed to a 11L–13D photoperiod did not molt into adults within 60 days after detachment. In addition, at a 10L–14D photoperiod, all of the engorged nymphs did not molt into adults within 60 days after detachment. These results suggest that the engorged nymphs enter morphogenetic diapause under short-day photoperiods. However, the diapause nymphs easily molted into adults when they were transferred into a 16L–8D photoperiod (Fujimoto unpublished). This suggests that the diapause nymphs gradually develop into adults even under short-day photoperiods. Thus, the intensity of the behavioural diapause in the engorged nymphs may possibly be weak.

At a 11L–13D photoperiod, both diapause and non-diapause nymphs occurred. This suggests that their critical photoperiod for the induction of diapause is
close to 11 hr. This critical photoperiod almost agrees with the day length of October in the Rokko Mountains. In the Rokko Mountains, thus, a part of the A. testudinarium nymphs that are active in autumn enter morphogenetic diapause and hibernate in the engorged state. Belozerov (1982) described that tick diapause provides two distinct advantages. The first advantage is synchronization of the life cycle with favourable seasons in the year. The second advantage is resistance to unfavourable conditions. The morphogenetic diapause of engorged A. testudinarium nymphs may also provide these advantages.

REFERENCES