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Biology of *Dermacentor silvarum* (Acari: Ixodidae) under laboratory conditions

JINGZE LIU[1,*], ZHANNIU LIU[2], YAN ZHANG[1], XIAOLONG YANG[1] and ZHIHUA GAO[1]
[1]*College of Life Science, Hebei Normal University, Shijiazhuang 050016, China;* [2]*Department of Biology, Xingtai College, Xingtai 054001, China; *Author for correspondence (e-mail: jzliu21@heinfo.net; phone: +86-0311-6268437; fax: +86-0311-6268313)*

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Abstract. The minimum life cycle of *Dermacentor silvarum* Olenev had a mean duration of 87.5 days (range 74–102 days) under laboratory conditions [(27 ± 1 °C), 70% RH, 6 L: 18 D]. The mean time in (days) for the different stages of its cycle was as follows: incubation period of eggs was 15.3 days; prefeeding, feeding and premoulting periods of larvae and nymphs averaged 5.5, 4.0 and 7.3 days, and 5.2, 5.0 and 14.6 days, respectively; prefeeding, feeding, preoviposition and oviposition periods of female adults lasted for 7.8, 4.5, 4.3 and 14.0 days, respectively. There existed a highly significant correlation between engorged body weight of females and egg masses laid ($r = 0.9877$, $p < 0.001$). The reproductive efficiency index (REI) and reproductive fitness index (RFI) in females were 11.09 and 9.58, respectively. No relationship between nymphal engorged body weight and resultant sexes was observed. Delayed feeding and non-oviposition (in June and July) existed in females, and low temperature (−10 °C) treatment for 45 days could terminate oviposition diapause. However, the egg masses laid by post-diapause females were significantly smaller than those laid by females engorged in March, April and May.

Introduction

Dermacentor silvarum Olenev, a three host tick, is widely distributed in North China (Deng and Jiang 1991), Russia (Kulik and Vinokurova 1983) and Mongolia (Das et al. 1989). Under natural conditions, they mainly reside in shrubbery, and can also infest broadleaf forest, conifer forest, as well as farmland (Liu et al. unpublished data). Domestic sheep and cattle are the main hosts for adult *D. silvarum*, whereas several mammals, including *Apodemus peninsulae* Thomas, *Rattus norvegicus* Berkenhout, *Mus musculus* Linnaeus, *Apodemus agrarius* Pallas, *Cricetulus triton* De Winton, *Mustela sibirica* Pallas, *Eutamias sibiricus* Laxmann are hosts for larvae and nymphs (Liu et al. unpublished data). This species is an important vector of Russian spring-encephalitis, *Babesia equi*, *B. caballi* and rickettsiosis, and can transmit these pathogens through the egg, and causes severe harm to human health and economic loss to livestock (Chen et al. 1998; Leonova et al. 1990; Deng and Jiang 1991).

In the light of the importance of *D. silvarum*, knowledge of its biology is very important. Unfortunately, there are no detailed biological data for this species. The present study was carried out to illustrate in detail the biology at various developmental stages of *D. silvarum* under laboratory conditions in the hope of providing basic background for further investigations.

Materials and methods

Collection and rearing of ticks

Adult ticks of *D. silvarum* were collected from naturally infested sheep in Xiaowutai National Natural Conservation Area (39°50′–40°07′ N, 114°47′–115°30′ E) of Hebei province, North China. Colonies of these ticks were reared and maintained in the laboratory. Unfed larva, nymph and adult were all fed on the ears of domestic rabbits. In order to facilitate the feeding and easy collection of engorged ticks after detachment, ticks were released into cloth bags glued on the ears of the rabbits. During tick feeding, the hosts associated with parasitic ticks were maintained at 25–27 °C and 50% RH, with a photoperiod of 6:18 (L:D) h. After detachment, ticks were collected. During the non-feeding period, the ticks were incubated in cotton-plugged glass tubes filled with one folded filter paper in an incubator [(27 ± 1 °C), 70% RH, 6 L: 18 D].

Observations on the biology of larvae and nymphs

In order to determine the prefeeding periods of larva and nymph, fifty newly hatched larvae and 50 newly emerged nymphs were brushed daily onto the ear of rabbit and observed for their feeding ability. Seven days after hatching/moulting, 300 unfed larvae and 100 unfed nymphs were weighed, then fed on rabbit, and checked twice a day. After engorgement and dropping off from the host, engorged ticks were collected, weighed and put into separate tubes. During their non-feeding period, they were maintained in an incubator. The prefeeding, feeding, and premoulting periods were determined. One hundred engorged nymphs were kept separately to determine their sexes after moulting.

Observations on the biology of adults

After moulting, 20 adults, including 10 females and 10 males were placed on a rabbit daily to determine whether they would attach and feed. Ten days after moulting, 50 females and 50 males, were weighed and put onto rabbits for feeding and were observed daily. The engorged females were collected, weighed, and put into the incubator. After all females engorged, males were manually removed from the host and weighed. Females were observed daily for their preoviposition period, oviposition period and egg masses laid. Daily deposited eggs were carefully separated from females, counted, and placed into separate glass tubes. Five hundred eggs were used for observing the incubation period. After hatching, the percentage of hatched larvae was calculated.

Treatment of engorged females with low temperature

In June, 50 females and 50 males were fed on rabbits, and were observed daily for their feeding. After detachment from the host, the engorged females were weighed and then put into glass tubes (plugged with cotton), then treated with low temperature in a refrigerator (−10 °C). Forty-five days later, the treated ticks were taken out and put in an incubator [(27 ± 1 °C), 70% RH, 6 L: 18 D]. The preoviposition period and egg masses laid of treated females were recorded.

Results

Life cycle of D. silvarum

Under the laboratory conditions, the average minimum life cycle for *D. silvarum* was 87.5 days (range 74–102 days), including prefeeding, feeding and premoulting of the larva (16.8 days), nymph (24.8 days), and the adult female (30.6 days), as well as the egg incubation period (15.3 days). The developmental duration obtained from various stages is presented in Table 1.

Feeding biology and changes of body weight

At the beginning of attachment, larva, nymph and adult fed slowly. As feeding progressed, their body size and weight increased gradually. During the later

Table 1. The duration of various developmental stages of *D. silvarum*.

Developmental stages	Period (d)	Number tested	Days, Mean ± SEM (range)
Egg	Incubation	500	15.3 ± 0.4 (13–16)
Larva	Prefeeding	300	5.5 ± 0.5 (5–7)
	Feeding	300	4.0 ± 0.6 (3–5)
	Premoulting	300	7.3 ± 0.3 (7–8)
Nymph	Prefeeding	100	5.2 ± 0.6 (4–6)
	Feeding	100	5.0 ± 0.6 (4–6)
	Premoulting	100	14.6 ± 0.7 (13–15)
Female	Prefeeding	50	7.8 ± 0.3 (7–9)
	Feeding	50	4.5 ± 0.5 (4–6)
	Preovipositon	30	4.3 ± 0.3 (4–6)
	Ovipositon	30	14.0 ± 1.7 (10–18)

stage of feeding (48 h for larva and nymph; 12–24 h for female after mating), larva, nymph and female fed rapidly and ingested large quantities of blood. The average weights of larva, nymph and adult before and after feeding are listed in Table 2. The weights of female nymphs were 57.1–73.3 mg, and those of male nymphs were 58.4–70.2 mg.

Oviposition

The oviposition data are shown in Table 3. Linear regression analysis revealed a highly significant positive correlation between the engorged body weight of females and the number of egg masses laid ($r = 0.9877$, $p < 0.001$) (Figure 1).

The daily egg masses laid varied with time (Figure 2). At the beginning of oviposition, the egg masses were small. A peak occurred 2–4 days after the onset of oviposition. From day 4, the daily egg masses declined steadily. Percentage of hatched eggs (hatched eggs/total eggs) was on average 86.4% (range 67–92%). The female reproductive efficiency index (REI) (number of eggs/engorged body weight) and reproductive fitness index (RFI) (eggs incubated to larva/engorged body weight) were 11.09 and 9.58, respectively (Table 3).

Diapause and low temperature treatment

In June, the female feeding period was significantly longer than in March, April and May ($p < 0.001$), the feeding period of the former was about 4.1 times

Table 2. Changes of body weight of larva, nymph and adult before and after feeding.

Developmental stages	Number tested	Body weight unfed (mg) (M ± SEM)	Body weight engorged (mg) (M ± SEM)	Weight ratio (engorged/unfed)
Larva	100	0.04 ± 0.00	0.42 ± 0.00	10.5
Nymph	100	5.68 ± 0.12	64.66 ± 4.71	11.3
Female	50	6.06 ± 0.99	461.4 ± 30.5	76.1
Male	50	4.78 ± 0.04	6.21 ± 0.13	1.3

Table 3. The characteristics of oviposition and egg hatches of *D. silvarum*.

Parameters	Number tested	Mean ± SEM (Range)
Engorged body weight (mg/♀)	50	461.4 ± 30.5 (369.2–544.8)
Egg mass laid (No/♀)	10	5116.7 ± 324.3 (4032–6090)
Preoviposition period (d)	30	4.3 ± 0.3 (4–6)
Oviposition period (d)	30	14.0 ± 1.7 (10–18)
Incubation period (d)	500	15.3 ± 0.4 (13–16)
REI	10	11.09
RFI	10	9.58

REI: Number of eggs/engorged body weight; RFI: Eggs incubated to larva/engorged body weight.

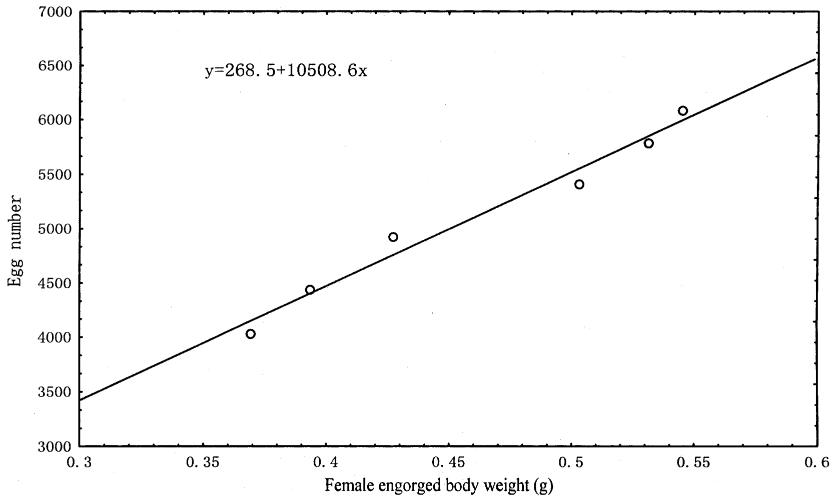

Figure 1. Relationship between egg masses and engorged body weight of *D. silvarum* (based on six females).

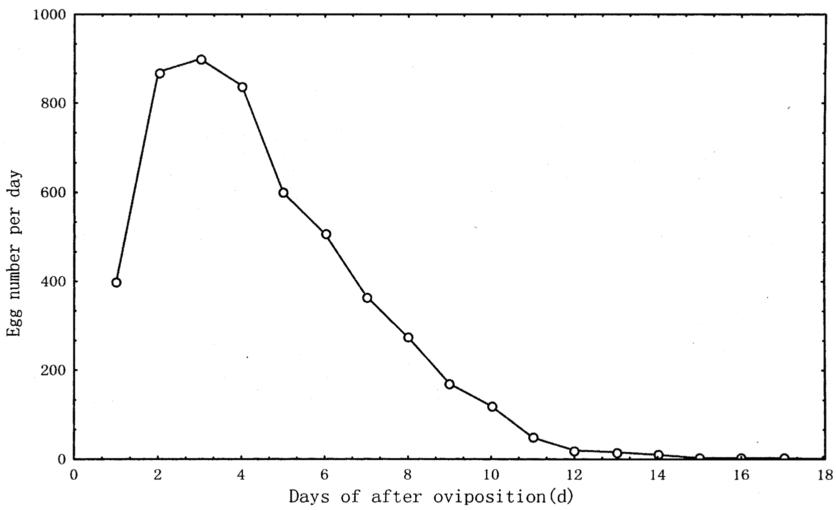

Figure 2. Mean daily oviposition of female *D. silvarum* (based on 10 females).

longer than the later. Comparing engorged females in July with females in March, April and May, there was no significant difference in engorged body weight ($p > 0.05$) (Table 4). After engorgement in July, the females did not lay eggs. After treatment of these females with low temperature for 45 days, they were placed in an incubator [(27 ± 1 °C), 70% RH, 6 L: 18 D] and they oviposited eggs 11 days later, although the egg masses were very low in numbers (326.4) (Table 4).

Table 4. Comparison of feeding, body weight and reproductive capacity (mean ± SEM) at different months of female.

Months	Feeding time (d) (Range)	Engorged body weight (mg)	Egg number (Range)
March, April and May	4.5 ± 0.5 (4–6)	461.4 ± 30.5	5116.7 ± 324.3 (4032–6090)
June and July	32.2 ± 6.8 (25–36)	458.7 ± 22.8	326.4 ± 68.7 (248–356)

Discussion

We reported some biological characteristics of *D. silvarum* for the first time. Under our laboratory conditions, *D. silvarum* can complete its life cycle in, on average, 87.5 days. Thus, they could possibly have at least *four* generations a year. However, they could only complete one life cycle in the laboratory. The reason is the failure of female to lay eggs. Field investigations by dragging from vegetation and detection from host reveal that the adults *D. silvarum* can only be found between later February and April. Apparently this species exists one generation per year under natural conditions and survives the winter as unfed adults (Liu et al. unpublished data).

We used several parameters to assess the reproductive biology of *D. silvarum*. The heavier the female body weight, the more eggs would be. A highly significant correlation existed between the female engorged body weight and the number of egg laid ($r = 0.9877$, $p < 0.001$). This relationship is also found in other tick species, *D. auratus* Supino (Jiang 1987), *Hyalomma schulzei* (Olenev) (Shoukry et al. 2000), *Haemaphysalis longicornis* Neumann (Liu and Jiang 1998), *Rhipicephalus bursa* Canestrini (Yeruham et al. 2000). The reproductive fitness index (RFI) was calculated by the number of eggs that hatched into larvae as a function of the engorged body weight (Chilton 1992). Because RFI takes into account the viability of eggs laid by females, it is more accurate and ecological importance to assess female reproductive fitness than REI. The RFI of female *D. silvarum* was 9.58.

It is reported in several tick species that heavier engorged nymphs become females, whereas lighter nymphs become males, which can be used as an index to predict sexes. In *Ixodes scapularis* Say, fully engorged nymphs moulted to females weighed between 3.8 and 6.4 mg, those nymphs that moulted to males weighed between 2.0 and 3.2 mg, body weights of engorged nymphs that become females were significantly greater than those of nymphs that become males (Hu and Rowley 2000). Similar results are also observed in *Hyalomma anatolicum* (Koch) (Arthur and Snow 1966), *I. ricinus* L. (Balashov 1972), *D. variabilis* (Say) (Hu and Rowley 2000), *Amblyomma inornatum* (Banks) (Gladney et al. 1977) and *Rhipicephalus bursa* Canestrini (Yeruham et al. 2000). However, our results indicate that no relationship between nymphal body weight and the resultant sexes of *D. silvarum* (58.4–70.2 mg for male; 57.1–73.3 mg for female) exists.

Diapause, a genetically determined state of suppressed development, is an important adaptive mechanism for dormancy during periods of unfavorable environmental conditions (Beck 1980). Two types of diapause occur in ticks, behavioural (host seeking) diapause and morphogenetic (developmental) diapause (Sonenshine 1993). The adaptive significance of tick diapause is primarily the synchronization of the life cycle with the favourable season of the year. In the current study, the feeding period in June for female *D. silvarum* was considerably longer than that in March, April and May, and the engorged female did not oviposite in July. These were indicative of a delay feeding diapause (behavioural) and a non-oviposition diapause (morphogenetic) existed in females. Whether other diapause type (s) exist in field conditions of *D. silvarum* needs to be further investigated. Photoperiod is believed to play the major role in regulating diapause in ticks and insects (Belozerov 1982; Sonenshine 1993). In many cases, photoperiodic signals not only induce diapause, but are also important in its maintenance and termination (Belozerov 1982). It is reported that moulting hormone can terminate larva diapause of *D. albipictus* (Wright 1969) and *D. sanguineus* (Sannasi and Subramonium 1972), *D. niveus* reproductive diapause (Wu et al. 1994). In the current study, although the egg masses laid were very low, our experiment proved that low temperature (−10 °C) treatment could terminate *D. silvarum* female oviposition diapause.

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