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Biology of *Ixodes (Pholeoixodes)* hexagonus under laboratory conditions. Part I. Immature stages

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ABSTRACT

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A rearing method for *Ixodes (Pholeoixodes) hexagonus*, the hedgehog tick, was established which enabled the life cycle of immature stages to be studied under laboratory conditions. Larvae were fed on Swiss mice and nymphs on the ears of New Zealand rabbits. The feeding time of the larvae and nymphs on both hosts was 4–17 days. Larvae moulted to nymphs 15–21 days after detachment from mice. The premoulting period was 13–26 days for newly emerged males and 15–27 days for females. Engorged nymphs which developed into males weighed less (5.64 ±0.91mg) than those that developed into females (6.019 ±88mg). The sex ratio (male: female) under laboratory conditions was 1:1.13.

INTRODUCTION

Ixodes (Pholeoixodes) hexagonus is one of the most widespread tick species in Europe (Morel 1965). The main hosts are hedgehogs (Arthur, 1953, 1963), foxes (Aubert, 1975; Harris *et al.*, 1978; Gilot *et al.*, 1985; Toutoungi *et al.*, 1991) and the Mustelidae (Gilot *et al.*, 1985; Mermod *et al.*, 1983; Toutoungi *et al.*, 1991). Domestic animals, *e.g.* cats and dogs, are often infested with this tick species (Arthur, 1953; Aeschlimann *et al.*, 1986; Liebisch *et al.*, 1989; Toutoungi *et al.*, 1991). In Switzerland, *I. hexagonus* is the most abundant tick species after *I. ricinus* (Aeschlimann *et al.*, 1965).

I. hexagonus is probably involved in the epidemiology of tick-borne encephalitis (Streissle, 1960; Krivanec *et al.*, 1988) and it was found to harbour *Borrelia burgdorferi,* the aetiologic agent of Lyme borreliosis (Liebisch *et al.*, 1989). Recently under laboratory conditions, *I. hexagonus* was shown to transmit *B. burgdorferi* transovarially and transstadially and to induce an infection in mice (Gern *et al.*, 1991). Despite the potential importance of *I. hexagonus* as a vector of

important human pathogens, comparatively little is known of the life cycle of this tick (Morel, 1965). In this paper we describe a method which has allowed us to establish and maintain a colony of *I. hexagonus* under controlled laboratory conditions and thereby examine the biology of the immature stages from larvae to newly emerged adults.

MATERIALS AND METHODS

The colony was initiated using larvae derived from an engorged female collected on a polecat *(Mustela putorius)*, and with nymphs removed from red foxes *(Vulpes vulpes)* from Switzerland. Larvae were fed on Swiss mice, while nymphs were fed on the ears of New Zealand rabbits. White mice were anaesthetized by intramuscular injection of Pentobarbital (Vetanarcol, Veterinary AG; Zürich) diluted with NaC1 solution (1/50), 0.1 mL per 1 g mouse weight. The rabbit ears were covered with a white cloth-sheath to prevent ticks from escaping. To prevent grooming, mice and rabbits were equipped with a collar as described previously by Graf (1978) for rearing *I. ricinus*.

Engorged larvae were grouped according to the detachment day, whereas engorged nymphs were maintained individually to prevent uncontrolled mating after moulting. Tubes containing ticks were kept inside a water container in trays at approximately 98% relative humidity. As *I. hexagonus* is an endophilic tick, all stages were kept in darkness, and at 22–23°C. For the control of the premoulting period in summer, 108 engorged larvae were kept at 30°C.

Daily examination of unfed and engorged larvae, nymphs and adults was undertaken to determine the duration of the prefeeding periods (defined as the time when newly hatched larvae and newly emerged nymphs were ready to feed), feeding periods (the time of attachment to hosts necessary to complete repletion), and premoulting+ moulting periods (the time interval between the day of detachment of engorged ticks and the emergence of the succeeding stage).

The Student's t test was used for statistical analysis. P values of less than 0.05 were regarded as significant.

RESULTS

Larvae

Prefeeding period: For the larval stage, a strong correlation was observed between the duration of the prefeeding period and the success in completing engorgement. When larvae were placed on mice one month after hatching, only 20% fed successfully, when they were placed 3–6 months after hatching, 50–70% completed their feeding. The percentage of success reached 95.7% with 11 month old larvae. After 12 months the degree of mortality of unfed larvae increased rapidly and only a small number of larvae attached to the host.

Feeding period: Larvae began to detach 4–5 days after placement on mice. Fig. 1 shows the daily drop-off rate for a total of 3389 larvae. 34.1% (11,256/3,389) of larvae were detached at the end of day 5 and 24% (816/3,389) were repleted at day 6. The midpoint (50%) of larval detachment occurred on day 6. All larvae had completed feeding by the 17th day.

To determine the success of feeding of the larval stage, we calculated the rate of engorgement of 500 larvae placed on two mice: 337/500 (67.4%) fed successfully.

Pre-moulting, moulting period: The incubation temperature was found to influence the duration of the premoulting and moulting periods. Fig. 2 shows the daily moulting rate of a total of 1194 engorged larvae maintained at 23°C and of a total of 108 larvae maintained at 30°C. When ticks were kept at 23°C the first nymphs emerged 15 days after drop-off. The 50% of the larvae (597/1,194) moulted 18 days after detachment from the host. The total period for ecdysis at this temperature was 27 days. At 30°C the premoulting and moulting periods shortened to 12–18 days, 50% (54/108) of these larvae moulted before day 14.

The moulting success of larvae was evaluated with 100 engorged larvae and we noticed that 80% metamorphosed into nymphs.

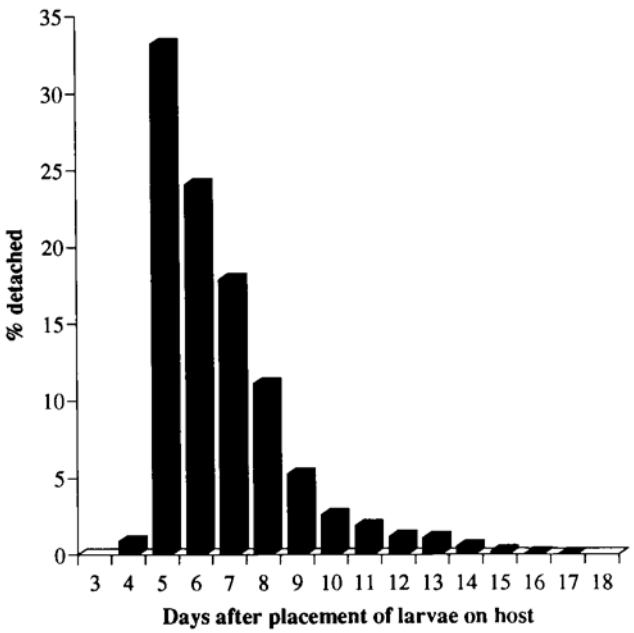

Fig. 1. Daily drop-off of 3,389 *I. hexagonus* larvae.

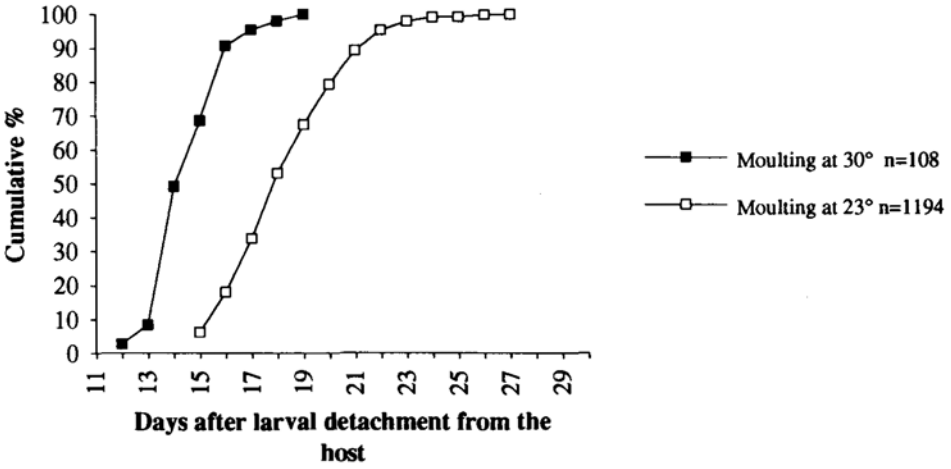

Fig. 2. Daily moult of larvae of *I. hexagonus*.

Nymphs

Prefeeding period: Nymphs fed successfully on rabbits 2 weeks after ecdysis.

Feeding period: Fig. 3 shows the nymphal drop-off rhythm of 278 engorged nymphs which developed into females and 253 engorged nymphs which became males. No significant difference could be observed in the duration of feeding period of female and male nymphs (t= 0.575, D.F.= 231, P= 0.566). The drop-off began 4 days after placement on hosts, 35.25% of female nymphs detached on day 5 and 19.06% on day 6. 42.3 % of male nymphs repleted at day 5 and 19.06% on day 6. The 50% of both groups of nymphs was situated at day 6. The feeding period of nymphs lasted for 17 days.

The feeding success of two batches, each of 100 nymphs placed on two rabbits, was 97% (194/200).

Weight of replete nymphs: The weight distribution of 323 fully engorged nymphs is shown on Fig. 4. It varied between 3.5 and 9.8 mg. The mean weight of engorged nymphs moulting into females was 6.019 ± 0.88 mg and that of nymphs which became males was 5.642 ± 0.91 mg. The difference between the two groups was significant (t=3.8, D.F.=321, P<0.005).

Nymphal to adult moulting: Fig. 5 shows the moulting dynamics of 98 female nymphs and 93 male nymphs. The premoulting period was shorter for males (13–26 days) than for females (15–27 days). The 50% (47/93) of engorged male nymphs

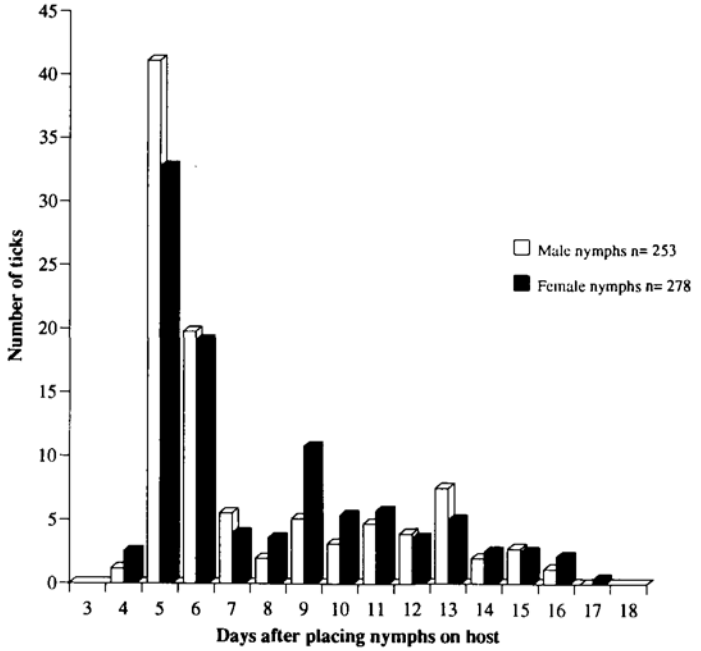

Fig. 3. Daily drop-off of *I. hexagonus* nymphs.

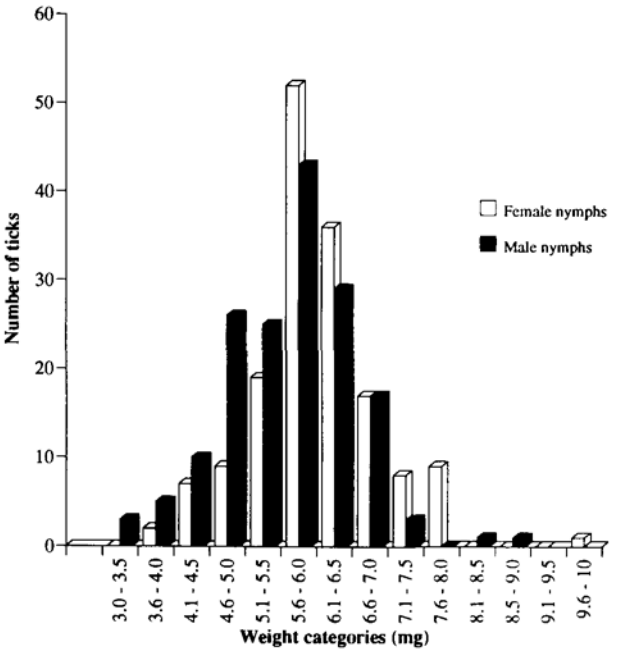

Fig. 4. Distribution of 323 engorged nymphal weights of *I. hexagonus*.

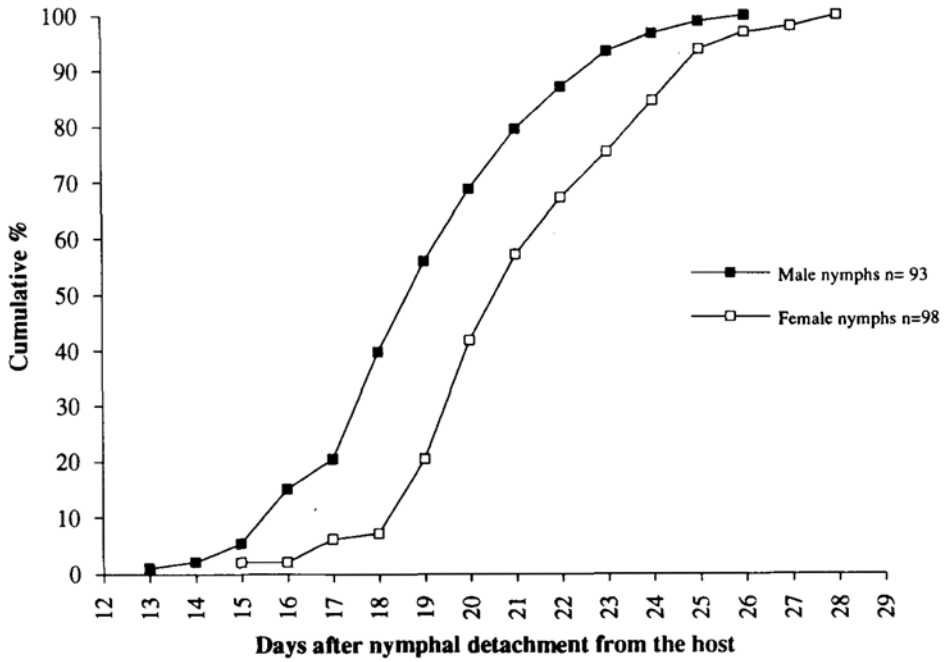

Fig. 5. Daily moult of engorged *I. hexagonus* nymphs.

moulted at day 18 and 50% (44/98) of engorged female nymphs moulted after 20 days. This difference was significant (t=3.02, D.F.=189, P=0.003).

The moulting success of 924 engorged nymphs was 88.2% (815/924). The sex ratio (male: female) of the newly emerged adults was 1:1.13.

The life cycle of the majority of the population of *I. hexagonus* was calculated by totalling the number of days of feeding and moulting of 50% individuals from the different stages and adding 15 days for hardening of nymphal integument. A minimum of 51 days was necessary for the development of larvae to adults stages with an average of 64 days. The percentage of 100 unfed larvae that became adults was 47% under laboratory conditions.

DISCUSSION

The rearing method described in this paper allowed us to obtain a sufficient number of ticks for the establishment of a *I. hexagonus* colony for biological studies under controlled laboratory conditions, and for use in other experiments on the transmission of microorganisms.

The rearing success from larvae to adults (47%) is relatively high if we compare it with other tick species reared in the laboratory. Graf (1978), using the same breeding conditions, reported a rate of 35–40% for *I. ricinus*. Smith (1972) obtained 38 adults of *I. canisuga* from 100 unfed larvae.

Under laboratory conditions, a minimum of one month for larval prefeeding period was observed, 50% of larvae began to feed 3 months after hatching, whereas 95.7% of 11 month old larvae attached to the host and completed their blood meal. This relative long larval prefeeding period shows that larvae do not feed directly after hatching. Arthur (1951) reported that the larval feeding period of *I. hexagonus* on hedgehogs was 3–6 days and the premoulting period of engorged larvae collected on hedgehogs and maintained at 22°C was 27 days. There is a little difference with our results (4–17 days for feeding period and 15–27 days for premoulting period). Arthur (1951) did not determine the day of larval fixation on the host and he did not explain the conditions under which he maintained the engorged larvae. High temperature diminished the duration of the moulting period of engorged larvae. This result confirmed the observations of Arthur (1951) and Honzakova (1971) who found that moulting from larvae to nymphs was prolonged by cold conditions and was diminished by high temperature. The effect of incubation temperature suggests that the time of metamorphosis may vary under natural conditions, and may be much longer in winter than in spring or summer.

There was no significant difference between the duration of the feeding period of male and female nymphs. The weight of engorged female nymphs was heavier than that of male nymphs. Similar weight difference was described for *I. ricinus* (Graf, 1978), and for other Ixodid species such as *Hyalomma. anatolicum* (Arthur *et al.*, 1966), *H. marginatum rufipes* (Knight *et al.*, 1978) and *Rhipicephalus evertsi* (Rechav *et al.*, 1977). However, the *I. hexagonus* male moulted earlier than the female nymphs, as reported for *I. ricinus* (Graf, 1978) and *R. evertsi* (Rechav *et al*; 1977) but not for *I. rugicollis* (Aubert, 1981). Under laboratory conditions, *I. hexagonus* took 51–105 days with an average of 64 days to develop from the larval to the adult stage. Arthur (1951, 1963) reported a period of an average of 62 days for *I. hexagonus* and 65–84 days for *I. festai*. Smith (1972) found a similar duration for *I. canisuga*. This period was longer for *I. rugicollis* (65–295 days) (Aubert, 1981). As rearing conditions were not uniform for the 4 species, the differences are probably not significant.

In conclusion, a period of three months was necessary for the development of *I. hexagonus* larvae to the adult stage under laboratory conditions (22–23°C, 98% relative humidity, darkness). The sexes of nymphs could be determined by the weight of engorged nymphs. The advantage of the rearing method described is the use of common laboratory animals.

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