Nymphal diapause and its photoperiodic control in the tick *Ixodes scapularis* (Acari: Ixodidae)

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Abstract. It is shown experimentally that the option between developmental diapause and non-diapause development in nymphs of *Ixodes scapularis* Say, 1821 (Middle Atlantic population) is determined by photoperiodic conditions according to a two-step photoperiodic reaction of short-day long-day type. Diapause arrest of development is induced by an impact of either long day upon unfed nymphs, or short day upon engorged nymphs, while non-diapause development completed in 2-2.5 months at 20°C needs the change from short-day to long-day conditions. Some ecophysiological aspects of mechanisms controlling seasonal development of ticks belonging to *Ixodes ricinus* complex are discussed.

The tick *Ixodes scapularis* Say, 1821 is the main vector of *Borrelia burgdorferi*, agent of Lyme borreliosis in eastern parts of the United States and the south-eastern part of Canada. The epidemiological and ecological aspects of *I. scapularis* have received considerable study (Spielman et al. 1985, Lane 1994, Piesman and Gray 1994). Numerous field data on its seasonal activity are available (Main et al. 1982, Lane et al. 1991, Fish 1993, Durden and Oliver 1999), and supplemented with limited data on seasonal development (Yuval and Spielman 1990, Lindsay et al. 1995, 1998). Moreover, attempts to simulate (model) its life cycle and population dynamics were also undertaken (Sandberg et al. 1992, Awerbuch and Sandberg 1995, Mount et al. 1997).

In spite of some serious contradictions in ecological data on seasonal activity of *I. scapularis* (see Main 1986, Spielman 1986), and a deficiency in experimental studies of its development and life cycle, it is possible to conclude, summarising all the available materials, that the life cycle of this tick is completed usually in 2-4 years. All its postembryonic stages are characterised by bimodal dynamics of their host-seeking activity (although unimodal dynamics occurs occasionally). They have the ability to overwinter both in unfed and engorged states. In these respects this Nearctic representative of the *Ixodes ricinus* species complex (Keirans et al. 1999) is rather similar to its Palaearctic relatives, *I. ricinus* and *I. persulcatus* (Belozarov 1976, Gray 1999). However, compared to them, *I. scapularis* has been much less studied in regard to seasonal adaptations (e.g. diapause) and extrinsic mechanisms that control them (e.g. photoperiodism).

Seasonal adaptations in *I. scapularis* are still unclear, although more than ten years ago Lane et al. (1991) noted that “the life history of *I. dammini* ... and the issue of diapause deserve research attention”. Since then only a few investigations of developmental and behavioural diapause in nymphs and larvae of *I. scapularis* have been conducted (Yuval and Spielman 1990, Lord 1995, Gray 1999), and of reproductive diapause in engorged females (Yuval and Spielman 1990, Lindsay et al. 1998). Behavioural diapause among unfed adults appears to be absent (Yuval and Spielman 1990). It is also suggested that there is an absence of “winter diapause stages” in this tick (McEnroe 1984). The role of photoperiod in this tick has not been studied, although there are assumptions that photoperiod controls its larval and nymphal diapauses (Yuval and Spielman 1990, Lord 1995) and affects behaviour of adult ticks (Goddard 1992).

Thus, there is a need for experimental research to better understand the life cycle of *I. scapularis* and the mechanisms of its seasonal regulation. Here we present the first results of an experimental study that confirms the presence of nymphal developmental diapause in *I. scapularis*, similar to *I. ricinus* and *I. persulcatus*, and its strong dependence upon photoperiods. The preliminary contribution on this subject was presented earlier (Belozarov and Naumov 2001)*.

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MATERIALS AND METHODS

Experiments on the effect of photoperiod upon nymphal development of *Ixodes scapularis* were conducted at the Biological Research Institute of St. Petersburg University (Old Peterhof). Ticks were obtained from the laboratory colony established at the E.I. Martinsonsky Institute of Medical Parasitology and Tropical Medicine (Moscow). The colony was begun from unfed adult ticks (Middle Atlantic population) received from the Parasite Biology and Epidemiology Laboratory, ARS, USDA (Beltsville, Maryland) in 1999. Two- to seven-week-old unfed nymphs (F_2 of the laboratory colony) were placed in February 2001 into two alternative photoperiodic regimens – long-day (LD, 22L:02D) and short-day (SD, 12L:12D) at 20°C. After two months they were fed on white outbred laboratory mice that weighed 20 g (under LD-conditions and 20°C). Two groups of unfed LD-nymphs and two groups of SD-nymphs (60 nymphs in each group) were used. The amount of engorged LD- and SD-nymphs was 52–80% and 35–53%, respectively. Engorged nymphs from each mouse were divided into two groups (numbering from 7 to 24 nymphs) which were maintained either in the same (as before feeding), or in alternative light-dark regimen at 20°C for six months. Off-host nymphs were maintained at an appropriate high humidity (95–98% RH). Apolysis, characterised by a cessation of leg movement and immobility of nymphs, and ecdysis (i.e. moulting itself), were recorded; the mean of apolysed or ecdysed nymphs from both replicates was calculated. On day 95, after the experiment was begun, one SD-group ($n = 15$) was transferred into LD-regimen, while the other group ($n = 23$) was left in the former SD-regimen (to check for possible reactivation after three-month maintenance of diapausing nymphs under SD-conditions). The complete scheme of experiments is presented in Fig. 1.

Means and standard deviations were computed for development of engorged nymphs from different photoperiodic regimens and compared using Student's *t*-test and modified Fisher's *F*-test (Plokhinsky 1967).

Some additional data from observations on larval and nymphal ticks of F_1 obtained at the Department of Entomology of the Martinsonsky Institute of Medical Parasitology are also presented in this paper for comparison.

RESULTS

Photoperiodic response of nymphs before and after feeding

Data on dynamics of engorged nymphs that began to apolyse and ecdyse at 20°C show that these processes depend on photoperiodic conditions of maintenance of nymphs both before and after feeding (Fig. 2).

Unfed nymphs maintained under SD-conditions, and subsequently transferred after engorgement to LD-conditions, revealed faster onset of apolysis and ecdysis (Fig. 2: lines 1a and 1e, respectively). Fifty percent of such nymphs apolysed in 45 days and ecdysed in 75 days after engorgement. The average pre-ecdysed period here was 79.4 ± 7.0 days. The duration of periods from beginning until completion of apolysis or ecdysis within a group was short (about 20–25 days). In

Fig. 1. Scheme of experiments on photoperiodic control of development and diapause in nymphal *Ixodes scapularis* maintained at 20°C. Unfed nymphs of initial stock (IST) were placed for two months into two alternative photoperiodic regimens – short-day (SD) and long-day (LD). After feeding on mice in LD-regimen, engorged nymphs were divided into two groups and transferred either to LD-regimen, or to SD-regimen. Some engorged nymphs from LD-SD group were returned to LD-regimen in three months after engorgement. Numbers in the right column correspond to the experimental variants (see Fig. 2). Engorged nymphs were checked regularly and apolysed and ecdysed specimens were recorded.

contrast, nymphs from SD-prefeeding regimen and retained after their engorgement in the same SD-conditions (line 4), revealed a stable diapausing arresting of development during the entire 6-month period after engorgement.

Unfed nymphs maintained under LD-conditions, and retained in the same LD-conditions after engorgement, had very slow and stretched-out development (Fig. 2: lines 2a and 2e) in contrast to nymphs maintained before feeding under SD-conditions (lines 1a and 1e). The difference between these groups is highly significant ($P \leq 0.005$), indicating the importance of day-length in unfed nymphs for their further development. Nymphs from the LD-preceding photoperiod began to apolyse in 98 days and to ecdyse in 118 days after engorgement (data for 50% of nymphs). The duration of the period between beginning and completion of apolysis or ecdysis within the group was more than 5 months. The average pre-ecdysial period in this group was 114.5 ± 35.8 days. However, nymphs transferred after engorgement into the SD-regimen revealed a strong tendency for developmental diapause (line 5), which was similar to nymphs that were maintained under SD-conditions, both before and after feeding (line 4).

Photoperiodic reactivation of engorged nymphs

Maintenance of engorged nymphs under SD-conditions not only enables the retention of stable developmental diapause (as described above), but also

Fig. 2. Dynamics of apolysis (1-3a) and ecdysis (1-3e) in engorged nymphal *Ixodes scapularis* at 20°C depending on photoperiodic conditions before and after feeding (experimental variants are enumerated according to the scheme in Fig. 1). 1 – nymphs maintained under SD-conditions before and under LD-conditions after feeding; 2 – nymphs maintained under LD-conditions both before and after feeding; 3 – nymphs maintained under LD-conditions before feeding, but transferred into SD-regimen for three months after engorgement and then returned into LD-regimen; 4 – nymphs maintained under SD-conditions both before and after feeding; 5 – nymphs maintained under LD-conditions before and under SD-conditions after feeding. Arrow with mark “5/3” shows the moment of transfer of engorged nymphs from SD- into LD-regimen (variant 3). Numbers at the level of dashed line designate duration of period (days) from nymphal engorgement up to apolysis and ecdysis for 50% of nymphs (means and both replicates). Abscissa – days after nymphal engorgement, ordinate – percent of apolysed or ecdysed nymphs.

evokes covert processes of their SD-reactivation. A three-month period of SD-treatment was enough to make engorged nymphs develop quickly after their transfer into LD-regimen (Fig. 2: lines 3a and 3e). They began apolysis in 35 and ecdysis in 57 days after transfer (data for 50% of nymphs), and the duration of the period between beginning and completion of any of these processes was only 22 days in this group. The pre-ecdysial period of engorged nymphs in this group was 65.0 ± 7.9 days after their transfer to LD-regimen. These results are comparable to nymphs maintained under SD-conditions before feeding and transferred into LD-conditions immediately after engorgement (lines 1a and 1e). But the average pre-ecdysial period in reactivated nymphs was 156.0 ± 8.9 days after engorgement. The difference in length of periods between nymphs from this and the two above considered groups are of great statistical significance ($P \leq 0.005$). This demonstrates the importance of both pre- and postfeeding photoperiodic regimens for the determination of development in nymphal *I. scapularis*. It also indicates the dependence of length of developmental arrest in engorged nymphs on their preconditioned state before feeding and on photoperiodic regimens after engorge-

ment. The arresting duration was dependent fairly directly on duration of postfeeding short-day impact.

Additional data on development of engorged larvae and nymphs

Development of engorged larvae of *I. scapularis* (F_1) under laboratory conditions at 20–25°C took 36 ± 3 days (50% of larvae) and 69 ± 15 days (100% of larvae). Moulting success was $57 \pm 14\%$ (for three groups). The rest of the larvae did not moult during 4 months of observation and either entered diapause or died. If 50 days are assumed as a limit for a non-diapause development, the percentage of larvae with developmental delay during 50 days varied from 9 to 42–45% within the three experimental groups.

Among engorged nymphs (F_1) development at 20–25°C took 79 days (50% of nymphs) and 107 days (100% of nymphs), with 78% successful moulting into the adult stage. In further experiments, using F_2 nymphs, non-diapause development at 20°C required 75 days (50% of nymphs) and 95 days (100% of nymphs) to moult into the adult stage. The mean mortality during this time was 18.5% among non-diapausing nymphs and only 8% (or less) among diapausing nymphs.

DISCUSSION

Our study confirms the presence of developmental diapause and its photoperiodic control in nymphal *Ixodes scapularis*. Data indicate that this control is based on a complex, two-step photoperiodic reaction combining two alternative photoperiodic responses – short-day (SD) and long-day (LD). These photoperiodic responses enable both induction and maintenance of diapause, as well as its elimination with further stimulation of developmental processes. In these regards *I. scapularis* is similar to the closely related Old World *I. ricinus* and *I. persulcatus* that belong to the same subgenus *Ixodes* s. str. (Belozеров 1995, 1998).

Based on information on seasonal photoperiodism in ixodid ticks (Belozеров 1982, 1999) and on photoperiodic responses of Eurasian *Ixodes* ticks, particularly *I. ricinus* and *I. persulcatus* (Belozеров 1995, 1998), mechanisms of photoperiodic control of diapause and development in nymphal *I. scapularis* may be presented as follows.

A consequence of two alternative photoperiodic reactions controlling diapause, is the possibility of two types of developmental diapause. Both of these are similar in arresting developmental processes (through preventing their initial stimulation), but differ by the mechanisms evoking and eliminating diapause. The first, SD-diapause, is a result of the direct response of engorged nymphs to SD-influences (according to norms of LD-reaction). This diapause is characteristic of engorged nymphs maintained in SD-regimens (Fig. 2: lines 4 and 5). The other, LD-diapause, results from an indirect response of engorged nymphs induced by LD-influences on unfed nymphs (according to norms of SD-reaction). This latter photoperiodic effect, being covert in unfed nymphs, is revealed after feeding as a slow gradual dynamics of moulting in nymphs placed into LD-regimen just after their engorgement (Fig. 2: lines 2a and 2e). In that case moulting is expressed in delays of variable duration before apolysis begins. Placing nymphs into SD-conditions after engorgement caused them to enter a stable diapause with no signs of development. Besides maintaining the diapause, SD-conditions cause simultaneously the opposite effect, namely its elimination (through reactivation, or “short-day sensibilisation” according to Zaslavsky 1972). Reactivated nymphs occur to be sensitive to LD-influences, and capable of apolysis and further

development after transfer into LD-regimen. This is due to LD-stimulation of moulting processes. This interpretation is confirmed by an additional experiment, in which LD-diapausing nymphs, maintained after engorgement for three months under SD-condition, were transferred into LD-regimen (Fig. 2: lines 3a and 3e). Apparently, short-day reactivation is possible in *I. scapularis* (similar to *I. ricinus* and *I. persulcatus*) not only in engorged nymphs with their overt diapausing state ensured by SD-influences, but also in unfed nymphs with their covert diapausing state induced by LD-influences.

It appears that nymphal diapause in *I. scapularis*, as well as in *I. ricinus* and *I. persulcatus*, results from elemental (basic) photoperiodic reactions of either SD- or LD-character (depending on type of diapause). However non-diapause development is enabled by complex, two-step photoperiodic reactions, where the first basic SD-reaction precedes the next, alternative LD-reaction. The first reaction represents a prerequisite and precondition of LD-response that initiate the beginning of apolysis and the further cascade of moulting processes leading to ecdysis.

All studied representatives of ticks from the *I. ricinus* species complex, both from Palaearctic (*I. ricinus* and *I. persulcatus* according to Belozеров 1995, 1998, and probably *I. kazakstani* according to Babenko and Gal'chenko 1976), and Nearctic (*I. scapularis*, this study) are characterised by common two-step photoperiodic mechanisms controlling seasonal development of nymphs. These complicated mechanisms of developmental regulation should be taken into account when analysing tick phenology, and when modelling tick population dynamics. Further studies of the mechanisms of developmental control in *I. scapularis* and *I. pacificus* in North America, which are the most important vectors of *Borrelia spirochaetes* and some other tick-borne pathogens, are needed.

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