Ticks and Tick-borne Diseases 8 (2017) 866–871

Contents lists available at ScienceDirect

Ticks and Tick-borne Diseases

journal homepage: www.elsevier.com/locate/ttbdis

Original article

# The influence of preprandial insemination on feeding and oviposition of *Ixodes persulcatus* females (Acari: Ixodidae) and some thoughts concerning mating strategies in ticks of the genus *Ixodes*

Inna Ioffe-Uspensky[1], Igor Uspensky[1,*]

*The Hebrew University of Jerusalem, Israel*

ARTICLE INFO

*Keywords:*
Prostriate ticks
Mating behavior
Copulation
Insemination
Oviposition
Subgenus *Ixodes*
*Ixodes persulcatus*

ABSTRACT

Prostriate ticks (subfamily Ixodinae, genus *Ixodes*) can copulate and the females can be inseminated before attachment to the host. In tests with *Ixodes persulcatus* females collected in the field and fed without males on the host, it was shown that this preprandial insemination is necessary and sufficient for successful engorgement and oviposition if female feeding took place in up to 1 month after collection. A 2-month period between preprandial insemination and female feeding was followed by a significant decrease in the proportion of normally engorged females and significant increase in egg mortality. If a small number of males were added to feeding females in this case, the number of normally engorged females increased but the egg mortality remained as high. Spermatophore destruction during the 2-month period is assumed to have a negative effect on the viability of eggs produced after additional (perprandial) insemination. Prostriate ticks are believed to be an intermediate group between argasid and metastriate ticks. Transition from nidicolous parasitism in argasid ticks to exophily (pasture parasitism) in metastriate ticks determines the change in mating strategy from off-host to on-host copulation. We review the available data concerning mating strategies in representatives of different subgenera of the genus *Ixodes* in the context of this evolutionary relationship.

## 1. Introduction

Copulation and subsequent insemination are the critical points for oviposition of ixodid tick females. In metastriate ticks, copulation of adult ticks and insemination of females take place on the host during female feeding. In contrast to this, in prostriate ticks (subfamily Ixodinae, genus *Ixodes*) copulation can occur either on or off the host and unfed females can be inseminated in the field before attachment to the host (Pomerantsev, 1950; Balashov, 1957, 1972; Arthur, 1962; Filippova, 1977). Males of many species in this genus have never been encountered on their hosts and are considered to lack the ability to feed (aphagy) (Balashov, 1972; Filippova, 1977). The reviews concerning the aspects of tick copulation and insemination mainly describe these phenomena for argasid and metastriate ticks, while only briefly touching upon prostriate ticks (Feldman-Muhsam and Borut, 1971; Oliver, 1974; Feldman-Muhsam, 1984). Only the latest reviews (Kiszewski et al., 2001; Kaufman, 2008) specifically address these phenomena in *Ixodes* ticks.

The best studied *Ixodes* ticks in relation to their mating strategies are the European wood tick *I. ricinus* in Europe and the deer tick *I. scapularis* (known at that time as *I. dammini*) in North America, both from subgenus *Ixodes*, which are important vectors of human and animal pathogens. In these species, unfed inseminated females were collected from vegetation and ticks *in copula* were found in the field as well as on the hosts (Graf, 1974, 1978; Gray, 1987; Yuval and Spielman, 1990; Yuval et al., 1990; Kiszewski and Spielman, 1999, 2002). The attraction of *I. ricinus* males to females of different feeding status (unengorged, semi engorged, fully engorged) and their ability to copulate was studied by Zemek et al. (2002).

As for the taiga tick *I.* (*Ixodes*) *persulcatus*, the most important vector of various pathogens in Eurasia (Uspensky, 2008, 2016; Korenberg et al., 2013), copulating adults were collected from vegetation by flagging (Babenko et al., 1979; Kolonin, 1987; Uspensky, unpublished observation) and unfed females were found to be inseminated (Repkina, 1973; Uspensky et al., 1978; Babenko et al., 1979). The ability of unfed males of the taiga tick to copulate and to inseminate unfed females was demonstrated in an experimental study (Uspensky and Repkina, 1978). When copulating, unfed males infected with tick-borne encephalitis virus or with *Borrelia burgdorferi* were able to infect unfed females with these pathogens (Chunikhin et al., 1983; Alekseev and Dubinina,

* Corresponding author.
*E-mail address:* igorusp.acarina@gmail.com (I. Uspensky).
[1] The present address: 16, Yustman Moshe St., Apt. 65, Jerusalem 9384208, Israel.

http://dx.doi.org/10.1016/j.ttbdis.2017.07.003
Received 4 February 2017; Received in revised form 28 June 2017; Accepted 2 July 2017
Available online 06 July 2017
1877-959X/ 

**Table 1**
Results of feeding and oviposition of *Ixodes persulcatus* females depending on male presence on the host [Mean ± S.D. (range)].

| Indices of tests | Test conditions | | | | No (%) females engorged | Feeding time (days) | Weight of engorged female (mg) | No eggs/female | REI | Egg mortality (%,approximately) |
|---|---|---|---|---|---|---|---|---|---|---|
| | Origin of ticks | No days[a] before test when males and females were separated | Ratio males: females on host | No females in test (No replicates) | | | | | | |
| C-1 | Laboratory colony | 30 | 1:1 | 10 (1) | 10 (100) | 7.8 ± 1.4 (5–9) | 378.5 ± 100.5 (196–490) | 3,230 ± 645 (2,025–3,910) | 8.6 ± 0.55 (7.6–9.2) | 3–5 |
| C-2 | –“ – | –“ – | 0:1 | 22 (2) | 0 (0) | | | | | |
| C-3 | Field population | 2–3 | 1:1 | 20 (2) | 19 (95.0) | 7.0 ± 0.8 (6–9) | 325.2 ± 47.1[a] (235–404) | 2,955 ± 503[a] (1,940–3,745) | 8.9 ± 0.39[a] (7.8–9.2) | 3–5 |
| T-1 | –“ – | –“ – | 0:1 | 24 (2) | 11 (45.8) | 8.2 ± 1.3 (6–10) | 295.4 ± 81.3[ab] (188–433) | 2312 ± 796[b] (930 – 3,335) | 7.8 ± 1.19[b] (4.9 – 8.5) | 5–10 |
| T-2 | – “ – | 30–32 | 0:1 | 24 (2) | 10 (41.7) | 7.1 ± 1.0 (5–8) | 252.9 ± 48.3[b] (165–306) | 1,774 ± 1,097[b] (270–3,475) | 7.0 ± 2.63[bc] (1.6–9.3) | 15–20 |
| T-3 | –“ – | 60–62 | 0:1 | 24 (2) | 3 (12.5) | 9.3 ± 3.6 (7–12) | 277.3 ± 158.5[ab] (175–393) | 1,582 ± 2,092[ab] (275–3,150) | 4.9 ± 4.6[abc] (1.6–8.0) | 40–85 |
| T-4 | –“ – | 60 | 1:3 | 12 (1) | 7 (58.3) | 9.6 ± 2.0 (7–12) | 314.4 ± 87.5[ab] (203–421) | 2,325 ± 1,147[ab] (825–3,690) | 7.4 ± 1.88[bc] (4.1–8.8) | 40–85 |
| T-5 | –“ – | 60–62 | 1:10 | 30 (3) | 7 (23.3) | 12.0 ± 3.2 (8–16) | 422.2 ± 125.9[a] (286–600) | 2,360 ± 989[ab] (1,245–3,715) | 5.6 ± 1.20[c] (4.3–7.3) | 40–90 |

Here and in Table 2, means followed by the same letters indicate no significant difference; means that are followed by different letters are significantly different.
[a] Days after molting (C-1 and C-2) or days after collecting (C-3 and all T tests).

1996a). Females infected after insemination with the virus could transmit the virus to the host while feeding, and pass the virus transovarially (Chunikhin et al., 1983). In all the above species males are able to feed on the hosts although their feeding is relatively short (Alexandrov and Yagodinsky, 1966). The ability of mating in unfed adult ticks was observed in several other species of *Ixodes* subgenus (*I. muris*, *I. petauristae*, *I. minor*, *I. eldaricus*) (Smith, 1944; Rajagopalan, 1963; Banks et al., 1998; Uspensky, unpublished observation).

The goal of the present study is to clarify whether preprandial insemination is always sufficient for normal feeding and oviposition of *Ixodes persulcatus* females and to compare the mating strategy of this species with those of representatives of different subgenera of the genus *Ixodes*.

## 2. Materials and methods

The tests described below were carried out with unfed adult ticks from the field population as well as with unfed adults of the first laboratory generation ($F_1$). The laboratory colony of *I. persulcatus* was initiated from unfed adults collected in May 1977 in Madona District of Latvia (then Latvian SSR). Ticks from the field population were collected in the same area in May 1978. The area was characterized by extremely high abundance of ticks of this species (Prisyagina et al., 1979; Babenko et al., 1979).

Collected or molted adult ticks were immediately separated by their sex. These ticks as well as females after engorgement or detachment were kept (males and females separately) in glass tubes where a gradient of humidity between 60% and 100% (Shashina and Ioffe, 1980) was maintained by distilled water slowly evaporating through sterilized cotton wool, fine sterilized sand and filter paper (wet chambers). Ticks were fed by placing them in linen bags glued to the back of rabbits: 10–12 females with varying numbers of males in a bag or without males at all. In total, 15 rabbits were used. The temperature in the laboratory was 22° to 25° C.

The methodology of the subsequent procedures and monitoring corresponded to that described earlier (Uspensky et al., 1975; Ioffe-Uspensky et al., 1997). The following parameters were registered during and after female feeding: duration of feeding, number and proportion of normally engorged females, weight of engorged females, length of the preoviposition period, and the number of eggs produced by a female. Batches of 3 fully engorged females taken in the beginning, in the middle and at the end of oviposition were checked under a stereomicroscope and the number of dead eggs was determined. The reproduction efficiency index (REI), i.e. the number of eggs laid by a female per weight of an engorged female (Balashov, 1957, 1972; Drummond and Whetstone, 1970), was computed.

The following versions of tests with ticks of the field population were conducted: females in 2-3 days, 1 and 2 months after collection put on the rabbit without males (T-1, T-2 and T-3, respectively), females in 2 months after collection put on the rabbit with males in different ratios (1 male:3 females in T-4 and 1 male:10 females in T-5). Three control groups of ticks were observed: virgin females from the laboratory colony that were fed together with males in 1:1 ratio (C-1) and without males (C-2), as well as females collected in the field and fed together with males in 1:1 ratio in 2–3 days after collection (C-3).

Attached females that engorged only slightly and did not increase their weight for several days were forcibly detached and weighed. Five females from the C-2, T-1, T-2, T-3 and T-5 groups (but only 2 females from T-4 group) were observed for their ability to oviposit and the viability of eggs was registered. The other females were dissected under a stereo-microscope in a drop of physiological solution to determine the state of their alimentary and genital systems and the degree of oocyte development using the stages proposed by Balashov (1972).

The statistical significance of differences between mean values of parameters registered in the tests was determined by paired Student's *t*-test. The difference was considered significant when $P < 0.05$.

## 3. Results

Up to 100% of control females from both the laboratory colony and the field population, which were fed together with the equal number of males (C-1 and C-3), normally engorged and laid eggs (Table 1). Females from the laboratory colony which were maintained without males before as well as during feeding (C-2) could not normally engorge