Published in Medical and Veterinary Entomology 8, issue 1, 101-103, 1994
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Some observations on mating and fertilization in the cattle tick *Boophilus microplus*

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Key words. *Boophilius microplus*, cattle tick, parasite, life-cycle, mating, fertilization.

The cattle tick *Boophilus microplus* (Canestrini) (Acari, Ixodidae) is the major ectoparasite of bovids throughout Australasia and South America, and is spreading through the African subcontinent. Economic losses are incurred through the direct debilitating effect of this one-host parasite on livestock, through damage to hides, and through the diseases transmitted, particularly babesiosis. Semiochemicals play a key role in guiding such diverse behaviours as host finding, attachment to the host, and feeding in ticks (Guerin *et al.*, 1992), and the importance of sex pheromones in these parasites has been well documented (Sonenshine, 1985). Fertilization is a *sine qua non* for engorgement in ticks (Oliver, 1974), so, at the University of Neuchâtel, we are currently investigating chemical signals implicated in the mating process of *B.microplus*. Although Nuñez *et al.* (1985) provide a detailed account of the life-cycle and morphology of the different life stages of *B.microplus*, particulars relating to the timing and nature of mating in this species are lacking. It therefore appeared appropriate to examine the behaviour of later development stages of *B.microplus*, which are clearly visible to the naked eye, in order to obtain some data on mating in this species and define its influence on engorgement.

B.microplus (Biarra, Queensland, organophosphorus resistant strain) has been reared under controlled conditions on young Simmental steers at Ciba-Geigy Agricultural Research Centre (Animal Health Subdivision), St Aubin for over thirty generations. The steers are held in pens (two to a 20 m^3 box) at 23°C, 60–70% r.h. and a 12:12 L:D cycle with photophase from 06.00 to 18.00 hours. Some 10,000 to 12,000 larvae between 2 and 4 weeks old were poured in a 30 cm line along the back from between the shoulders of the restrained steer in the forenoon of day 1. Details of the developing population were obtained by removing ticks before noon with forceps from day 12, when individual nymphs are clearly visible to the eye, and at regular intervals thereafter. Measurements (length and breadth) of females ($n \geq 10$) were made on a haemocytometer under a stereomicroscope after clearing individuals of residual hairs. To determine the time of day at which fertilization occurs, females were additionally sampled at hourly intervals during the scotophase of days 15 and 16. The receptaculum seminis was dissected out and smeared on a microscope slide to check for sperm.

Observations on mating were made on *B.microplus* adults attached to rabbits' ears (New Zealand white breed). For this, replete nymphs were harvested from the steer on day 12 and allowed to moult overnight in an incubator at 23°C and 90% r.h. Thereafter, males and females were maintained separately in bags on rabbits' ears where more than 75% attached. Newly moulted females from the incubator were also allowed to attach to a steer within two foam-rubber arenas (6 × 6 cm inside and 3 cm high) which were glued to the shaved hips of the animal before infestation with larvae; males of the same population found wandering over the surface of the host were prohibited from entering these arenas by a gauze covering with a press fastener (Velcro®) attachment. Observations on mating were made on the rabbit's ear by removing a male with forceps, placing it in the vicinity of a female and viewing with an operation microscope equipped with a coaxial cold-light source. Video records were simultaneously obtained with a colour CCD video-camera mounted on the microscope.

Boophilus microplus females require on average from 19 to 21 days on the steer to complete the cycle from larva to engorged adult in the controlled conditions described. On day 12, 29% of ticks have moulted into males (ninety-one adult males and one adult female among 311 ticks sampled). Almost all males have moulted and attached to the host, venter-to-venter with a pharate female, by day 13 (35/38 females sampled had a mate). The adult females moult on day 13 (14/38 sampled) and this is completed by day 14 (one unmoulted nymph in sixty-one sampled).

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Male moult precedes that of females not only on the host, but also amongst those allowed to moult in the incubator. Nuñez *et al.* (1985) also noted this protandry on the host. As female *B.microplus* moult *in situ*, with the female even retaining the exuvium attached to the capitulum, the pairing undertaken previously by the males is not disrupted. Wilkinson (1954) has already remarked on the ability of adult male *B.microplus* to pair with replete female nymphs.

Fertilization commences some 72 h after the appearance of the first females, i.e. the number of fertilized females increases during the first half of the scotophase of day 15 (Fig. 1). This is followed by an increase in the mean size and weight of females sampled in the forenoon of day 16. Onset of fertilization coincides with the first observations of male movement on the steer, and although this continues until the end of the infestation, it peaks each day during the first half of the scotophase. The percentage of fertilized females increases through the scotophase of days 16 and 17 to reach 100% on day 18. Drop-off proceeds likewise over 3 days, i.e. during the nights of days 18, 19 and 20. Female *B.microplus*, fertilized 3 days after moult, require an additional 3 days to complete engorgement to weights ≥200 mg. This coincides with the interval reported by Nuñez *et al.* (1985) between the appearance of females on the host and the first detachment of *B.microplus* in the field.

Although 2-day-old adult male *B.microplus* will show no interest in freshly re-attached females on a rabbit or steer, this changes dramatically after 24 h. Thereafter, females retain this capacity to activate males for several days. The male will quickly mount the female, investigate the dorsum with forelegs and rostrum, pass to the female venter via the idiosoma, insert its mouthparts into the female gonopore and transfer the spermatophore some 10 min later. Once the male has reached the caudal zone, the female cooperates by lifting her body on its legs at an angle to the host, thus facilitating male passage to the venter. This characteristic mating routine leading to fertilization is similar to that already described for a number of pro- and metastriate tick species (Graf, 1978; Sonenshine, 1985). Selective masking of chemosensilla on the tarsi and palps of male *B.microplus* disrupts mating behaviour, demonstrating that female-produced semiochemicals also play a key role in this species in guiding male behaviour (Guerin *et al.*, 1992). After copulation, the male proceeds to re-attach venter-to-venter with the female and apparently undertakes a brief feed as evidenced by excretion of a small quantity of fluid some minutes later, which quickly crystallizes. All semi-engorged or engorged females sampled on the host are accompanied by a male. Although females held for an extended period devoid of males on the rabbit and steer do undergo a colour and even a slight size change with time, they never proceed to engorgement, confirming observations by Nuñez *et al.* (1985)

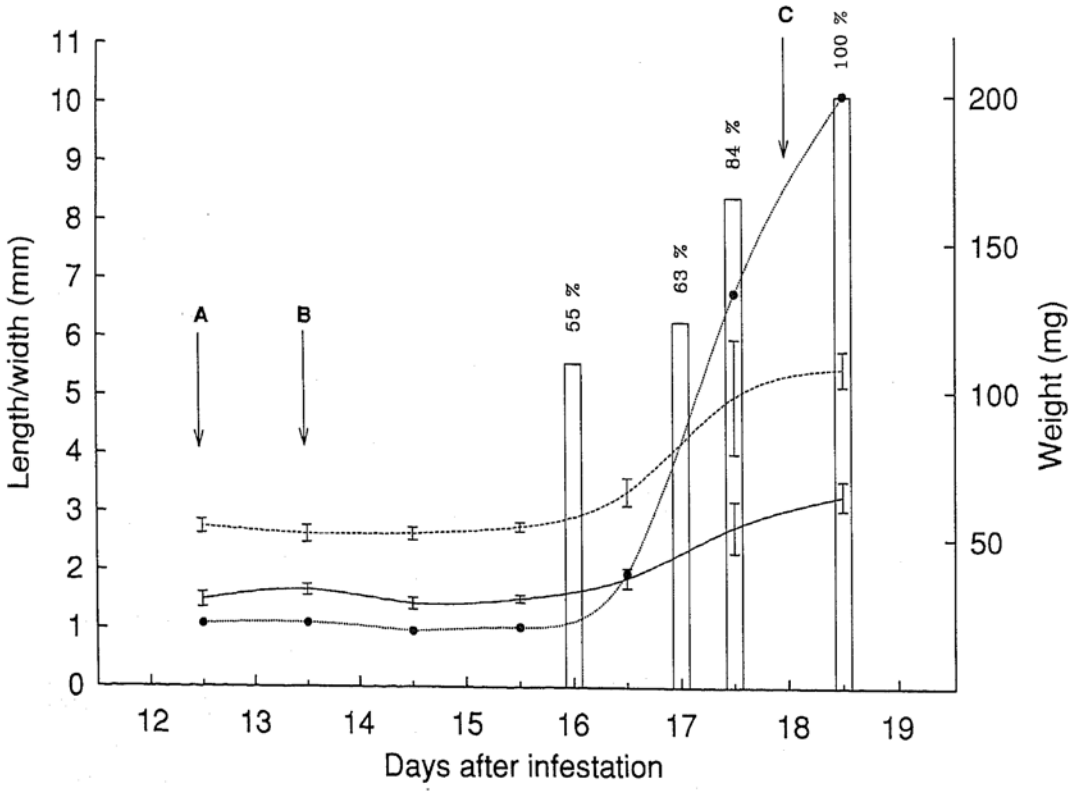

Fig. 1. Development of *B.microplus* infestation on a young steer held in a climatized box. Moult of nymphs into adult males occurs on day 12 (arrow A), and that of females on day 13 (arrow B). Female lengths (----), widths (——) and weights (●·····●) all increase after onset of fertilization on night 15, and this continues apace with the increase in percentage females fertilized through nights 16 and 17 (columns). Means ±standard deviation are presented for length and width measurements on individual females ($n \geq 10$), whereas weight change of individual females was calculated from the combined weight of ten individuals per sampling occasion. The first fully engorged females drop from the host in the seventeenth night (arrow C) but drop-off peaks in the nineteenth night; marks on abscissa opposite day numbers represent 00.00 hours.

The behaviour of adult male *B.microplus* is influenced by two stages of female development, namely by pharate and maturing virgin females. Sampling ticks in the host's pelage shows that newly moulted males attach preferentially near pharate females. We must assume that, some 72 h later, the maturing female, shown here as being capable of inducing copulation behaviour in males placed nearby, can likewise induce detachment by its male partner for copulation as in other metastriate tick species. Experiments on a rabbit's ear show that mature males walking over the host are arrested and stimulated to copulate with unmated females of the same age or older. The increasing number of males witnessed walking on the host as the parasite population matures is a consequence of their desertion by the engorged female; only males remain at the end of the infestation. Male *B.microplus* can mate more than once, and mean survival time is estimated to be over 40 days (Thompson *et al.*, 1980). We have also observed an increase in size with maturation in males. In this one-host tick species the male's ability to recognize and copulate with later-maturing virgin females is probably important under field conditions, where development of the parasite population on the host covers a wider time span than that recorded here in the confines of a climatized pen. This description of timing and nature of mating in *B.microplus* has set the foundation for studies on the stimuli influencing sexual responses of males, and behaviourally active extracts of pharate and unmated females have already been obtained (de Bruyne, unpublished).

Acknowledgments

We are grateful to the Hasselblad, Roche, Sandoz and Swiss National Science Foundations, the Ciba-Geigy-Jubiläums-Stiftung, Schweizerische Mobiliar, and the Swiss Office for Education and Science for funding research on the chemical ecology of ticks at the University of Neuchâtel.

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