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ABSTRACT: Few female Amblyomma triguttatum triguttatum caught from the vegetation had copulated. The age of unfed females did not affect mating. Males lost weight after being on the host and there was no evidence that they fed. Unfed males performed a mean of 2.7 matings. During copulation they introduced only the cheliceral digits into the female genital aperture. Copulation lasted longer after the first mating. The initial pre-copulation period of unfed males was longer at 25°C than at 30 or 35°C. Unfed adults released pheromones with inter- and/or intra-sex attraction. It is suggested that copulation normally takes place around the time of female attachment to a host; this provides the highest fertility of the eggs.

RÉSUMÉ: Quelques femelles d'Amblyomma triguttatum triguttatum capturées dans la nature avaient copulé. L'âge des femelles non gorgées n'affecta pas l'accouplement. Les mâles perdirent leurs poids après passage sur l'hôte et il n'y avait aucune évidence qu'ils s'étaient gorgés. Les mâles non gorgés réalisèrent une moyenne de 2,7 accouplements. Au cours de l'accouplement ils introduisirent uniquement les appendices chélicéraux dans le réceptacle génital femelle. La copulation dura plus longtemps après le premier accouplement. La période de pre-copulation des mâles non gorgés était plus longue à 25°C qu'à 30 ou 35°C. Les adultes non gorgés sécrètent une ou des phéromones d'attraction inter et/ou intrasexuelle. Il est suggéré que la copulation prend normalement place approximativement durant le temps où la femelle est attachée sur l'hôte ; cela permet la plus grande fertilité des œufs.

INTRODUCTION

Only 3 species of three-host ticks of the Metastriata group of Ixodidae are known to produce elongate spermatids prior to feeding. Oliver & Stone (1983) described this with Aponomma hydrosauri (Denny) and Ap. concolor Neumann, and Guglielmone & Moorhouse (1983) with Amblyomma triguttatum triguttatum Koch. However Andrews & Bull (1981) reported that both male and female Ap. hydrosauri needed to feed for at least 5 days before mating behaviour commences. Unfed males of Am. t. triguttatum were able to fertilize unfed females off the host, but the eggs laid by females which had copulated 18 days before the onset of feeding had a lower

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viability than those from females which had copulated on the host (GUGLIELMONE & MOORHOUSE 1983). This was possibly due to death or lower viability of spermatozoa retained for a period in the seminal receptacle of the female.

The present work was designed to further investigate fertilisation in *A. t. triguttatum*. In this article *Aponomma* is abbreviated to *Ap.* and *Amblyomma* to *Am.* to avoid confusion.

**MATERIALS AND METHODS**

**Copulation of unfed females off the host under natural conditions.**

Fifty carbon dioxide traps (GUGLIELMONE & MOORHOUSE 1985), where 'dry ice' in a container was placed in the centre of a 90 cm square of white flannelette on the ground, were set up between November 1981 and February 1982, and 30 during the same months in 1982-83. All females approaching the traps were collected, and separated into 2 groups; those without males and those carrying males. Both groups were dissected to show the presence of spermatozoa.

**The influence of age on the sexual behaviour of unfed adults.**

Nine males, 1-3 days old, were each put with a 1-day-old female, and a second group of 9 males, 1-3 days old, were put with 45-day-old females. Analysis for differences between the mean precopulation periods was by the 't' test.

Eight unfed 1-day-old females were held with 8 unfed 55-day-old males for 24 h and 4 unfed 55-day-old females were held with 4 unfed males of the same age for 24 h.

All ticks were held in 5 cm diameter Petri dishes and maintained at 30°C and 2-3 mm Hg saturation deficit in darkness. Implantation of a spermatophore was taken as the indicator of copulation.

**Copulation by unfed males.**

A group of 27 males, maintained as above, were followed for 60 days. Throughout this period each male was held with the same female, as the females are known to be receptive to more than one mating (GUGLIELMONE & MOORHOUSE, 1983). The experiment was concluded when 10 days had elapsed after the last spermatophore was deposited. Ten of the males were then released onto a calf and allowed to attach for 12 days, unless they detached earlier. After being on the host they were again held with females and observed for additional matings.

**The effect of temperature and saturation deficit.**

Experiments were carried out at 20, 25 and 30°C each with 3 levels of saturation deficit (2-3, 8-9 and 16-17 mm Hg). Ten pairs of ticks (1-3 days old) were allotted to each treatment (a total of 90 pairs). The age of the males at their first copulation was recorded and results were tested for significance using a two-way analysis of variance.

**Male attachment.**

Two groups of 10 males aged 45 days were each marked by cutting off different legs at the metatarsal level, as used by NORVAL & CAPITINI, 1974, and individually weighed. The ticks were then released onto a calf, and restrained within plastic capsules (8 × 8 cm), covered with muslin and cemented to the host. The males were checked twice daily for attachment or detachment. One group was held along with females and the other group without. When detachment occurred, or after 12 days on the host, they were weighed and dissected to obtain samples of gut. Perl's Reaction, preceded by treatment with 30% hydrogen peroxide (PEARSE, 1972), was performed on squash preparations of their guts to test for haemoglobin.
Adults' pheromones

Petri dishes, 5 cm in diameter, were divided into quadrants. Section 1 contained filter paper (1 x 1.5 cm) that had been in contact with either male or female ticks for 7-10 days; the other 3 quadrants were covered with clean filter paper. A tick was considered attracted when the time spent in contact with the paper in section 1 was more than a quarter of the total time spent with all the filter papers.

Ten ticks of each sex were observed individually for 10 minutes after being placed in the centre of the Petri dish. The test was repeated 4 times, with section 1 having a different orientation each time. All tests were conducted under artificial light at room temperature (approximately 22°C).

RESULTS

Copulation by unfed females off the host under natural conditions.

Of 41 females caught before contacting males on the traps during the summer of 1981-82, 3 (7 %) had been fertilized, while none of 20 caught during 1982-83 were fertilized.

In the second group where 16 females were caught after contacting males, all had copulated. Females of this group were able to walk with males clinging to them.

The influence of age on the sexual behaviour of unfed adults.

No significant difference (P > 0.05) was found between the mean of the initial pre-copulation of unfed males held with newly moulted or mature females. The mean pre-moult periods (days) and standard deviation were 16.4 ± 6.326 for males held with newly moulted females and 18.7 ± 8.059 for males held with 45-day-old females. Moreover, all newly moulted or 55-day-old females held with 55-day-old males for 24 h had spermatophores implanted.

Number of copulations performed by unfed males.

The results are presented in Table 1. The males performed a mean of 2.7 copulations. Only 3 of the 10 males put on the calf attached, 1 for 1 day, another for 9 days and the third for 11 days; no additional copulations occurred after being on the host. During copulation, *Am. t. triguttatum* males thrust only the chelical digits into the female's genital aperture. The duration of coupling ranged from 15 min to 48 h, increasing after the first mating.

<table>
<thead>
<tr>
<th>Number of copulations performed</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of males</td>
<td>5</td>
<td>7</td>
<td>11</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Period between consecutive mating (range in days)</td>
<td>1-30</td>
<td>1-24</td>
<td>1-5</td>
<td>10-15</td>
<td>1-5</td>
<td></td>
</tr>
<tr>
<td>Number of males included in the range</td>
<td>22</td>
<td>15</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>

* In darkness at 30°C, 2-3 mm Hg of saturation deficit.

The effect of temperature and saturation deficit.

The interaction of temperature and saturation deficit was of no significance (P > 0.05). The temperature affected the initial pre-copulation period (P < 0.05). It was longer at 25°C (mean and standard deviation = 27.8 ± 7.076 days) than at 30°C (16.7 ± 3.010) or 35°C (15.9 ± 3.814).

Male attachment.

Two males of the group held without females and 8 from the group held with females attached for 1-5 days. However these males lost weight while on the host. The mean of the initial weights of males held alone was 11.4 ± 2.660 and, after being on the host it was 10.6 ± 2.525. The mean of the initial weights of males held with females was 12.4 ± 2.420 and it was 11.3 ±
after being on the host. All Perl’s tests for haemoglobin were negative and no trace of blood was found in the guts when males were dissected.

Adults' pheromones.

Nine out of 10 males were attracted to filter papers that had been in contact with unfed females (P < 0.01). A similar result was found with males exposed to papers in contact with males. On the other hand, 7 out of 10 females were attracted to papers that had been in contact with males; this was not statistically significant (P > 0.05). All females were attracted to papers that had been in contact with unfed females (P < 0.01).

DISCUSSION

Few Am. t. triguttatum females caught from the vegetation had been fertilized. This was unexpected since the age of the unfed females was not a barrier for mating and in the laboratory they produced pheromone/s that attracted unfed males. The attraction to the host (simulated in this situation by a carbon dioxide source) favours sexual encounter and copulation. The sex drive must provide the attraction for males, since the evidence suggests that they do not feed.

It is suggested that Am. t. triguttatum has adopted a reproduction strategy that generally precludes copulation in the absence of a host. If copulation occurs long before female attachment to a host then the viability of the eggs is reduced (GUGLIELMONE & MOORHOUSE 1983). The fact that females were able to walk with males clinging to them suggests that females may be able to attach while mating is in progress.

The failure of Am. t. triguttatum males to gain weight, even when attached to a host, is unusual for a Metastriata tick. Possibly Am. t. truguttatum males merely anchor to the host, especially in the presence of females, and the time they remain attached could be related to a resting interval between copulations. Males of other Metastriata such as Hyalomma excavatum (Koch) showed a 2-fold increase in weight after being on the host (RECHAV & OPPENHEIM 1969), while Amblyomma hebraeum Koch increased their initial weights by 20% (NORVAL & CAPITINI 1974). BALASHOV (1972) provided evidence of weight increases between 50 and 100% in males of 4 species of Metastrata ticks. Similarly RECHAV & KINIGHT (1983) showed that the males of Rhipicephalus oculatus Neumann almost double their weight over 6 days of feeding.

Feeding of male Am. t. triguttatum is probably of no importance in the reproduction of this tick, since unfed males were capable of mating more than once without being on the host. The number of copulations performed by unfed male Am. t. triguttatum was higher than that recorded with unfed I. ricinus L. (GRAF 1978). Male of this latter species copulated a maximum of 4 times and the majority only twice, compared with a maximum of 6 matings by male Am. t. triguttatum, while the majority copulated 3 times.

The initial pre-copulation period at 25°C was longer than at 30 or 35°C, and may be related to a slower rate of spermatogenesis at lower temperatures. The lack of influence of saturation deficit supports the view that it plays no role in spermatogenese or mating behaviour. No other records of this aspect of sexual behaviour of male ticks from species not requiring to feed prior to mating could be found. However, BALASHOV & GOROSCHENKO (1960) stated that at 25°C 3 species of Ornithodoros and Argas persicus (Oken) usually copulated 4-6 days after moulting. BALASHOV (1956) considered that I. ricinus males copulated immediately after moulting, but GRAF (1978) pointed out that there was an interval of 15 days between moulting and the first copulation. These examples suggests that spermatogenesis and maturation is accomplished more quickly in these argasids and ixodid ticks than in Am. t. triguttatum at 25°C. GRAF (1978) also observed that the duration of the process of copulation by I. ricinus increased after the first copulation.

Thrusting of only the cheliceral digits into the female’s genital aperture, as observed with Am. t. triguttatum, is the usual pattern in the Metastrata (FELDMAN-MUHSAM & BORUT 1971). The only
apparent exception is *Amblyomma dissimile* Koch (Bodkin 1918), but the description of penetration in this paper is not really clear and as Feldman-Muhsam & Borut (loc. cit.) comment "we should be surprised if the pattern of copulation in this genus should differ from that of other Metas­triata".

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