[SHORT COMMUNICATION]


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**INTRODUCTION**

Copulation in ticks is completed by transfer of a spermatophore following insertion of the male mouthparts into the female genital aperture. It is known that females receive mechanical (insertion of the male mouth parts and spermatophore) and chemical (the salivary gland secretion and spermatophore components) stimuli during the copulatory behaviour. Argasidae and Prostriata species are able to copulate with or without feeding at the adult stage. However, only a potent male (completely fed) can copulate with a receptive female (partially fed) on the host in most Metastratiata (Ixodidae: except for *Ixodes*) species. Since physiological changes are induced in female ticks after copulation, the copulatory stimuli appear to contain important factors regulating their reproduction. That is, we are able to consider that copulatory stimuli may affect in Metastratiata more directly than other ticks. There are many studies on copulatory stimuli in ticks, especially changes of the female feeding activity and status after artificial treatments in Argasidae and Metastratiata (Aboul-Nasr and Bassal, 1972; Aeschlimann and Grandjean, 1973; Balashov, 1972; Diehl et al., 1982; Feldman-Muhsam, 1973; Galun and Warburg, 1967; Germond and Aeschlimann, 1977; Graf, 1978a, b; Oliver et al., 1975; Papas and Oliver, 1971, 1972; Rechav, 1968; Sonen-shine, 1967), however, most of these effects were incomplete and their functions have not been explained completely. Effects of natural and artificial stimuli in cuticular plasticization, which is an important event enabling a great deal of feeding, were observed in the metastriate female *Haemaphysalis longicornis* Neumann (Okura et al., 1996, 1997). In the present study, engorgement status and oviposition of females after some experimental treatments were preliminarily observed in order to elucidate functions and mechanisms of copulatory
stimuli in female *H. longicornis*.

**MATERIALS AND METHODS**

Adult *H. longicornis* were collected by dragging in pastures of Kuju Highland, Oita Prefecture, and males and females were fed separately on ears of laboratory rabbits until the males became potent (for 7 days) and females sexually receptive (for 5 days). The male accessory genital glands were removed from completely fed males in cold physiological saline (0.8% NaCl) including 1 mM phenylmethylsulfonyl fluoride as a protease inhibitor. A potent male and a receptive female were allowed to begin copulation on the host and the spermatophores were collected from the male genital aperture immediately after its formation. These females were used as individuals stimulated only by the male mouthparts (MP female). The accessory glands and spermatophores were homogenized separately in the same physiological saline. The homogenates were centrifuged at 3000 g for 10 minutes and the supernatants used immediately for injection experiments.

Approximately 1.5 accessory glands or spermatophore equivalents per 3 µl of the supernatant were injected into the haemocoel untreated receptive virgin females and MP females with a glass micropipet (about 100 µm external diameter). Untreated females injected with 3 µl of the same physiological saline were used as controls. That is, six groups, only insertion of the mouthparts (MP female), only injection of the accessory gland extract (AG female) or spermatophore extract (SP female) and combination of insertion of the mouthparts with injection of the accessory gland extract (MP+AG female) or spermatophore extract (MP+SP female) and control were investigated.

Idiosomal length, width, and depth (the length between dorsal and ventral surfaces) of all females were measured with a 0.1 mm precision caliper immediately after and 48 hours after treatment. *H. longicornis* females engorge and detach from the host within 48 hours after normal copulation therefore females were forcibly detached from the host, and maintained at 28°C, 90 RH. The number of eggs laid in MP, AG, SP, MP+AG and MP+SP females were counted and all eggs were preserved for one month under the above conditions.

In accordance with Yano *et al.* (1989), the body volume of ticks was estimated using the stereometry of a spheroid ($\pi/6 \times \text{length} \times \text{width} \times \text{depth}$). The relative body volume (RBV) was also calculated: the body volume 48 hours after treatment divided by the volume just after treatment. The RBV was taken as a measure of the degree of feeding. A difference in the effects of the treatments on the RBV was analyzed by one-way ANOVA (Analysis of Variance). If a significant difference was detected, further analyses were carried out with the Least Squares Mean.

Furthermore, a parthenogenetic race of *H. longicornis* is known to exist, but they engorge for only 5 days, whereas, the two gender race becomes receptive (partially fed). Therefore, the parthenogenetic race can be distinguished from the two gender race. In addition, eggs oviposited were nonviable in the present study. So, it is ensured that complete copulation did not occur and the parthenogenetic race is absent from the present experiments.

**RESULTS AND DISCUSSION**

The RBV (mean±S.E.) of the control female, MP female, AG female, SP female, MP+AG
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female and MP+SP female were 1.04±0.48, 1.90±0.51, 2.88±0.32, 3.45±0.51, 2.77±0.45 and 2.58±0.43, respectively (Table 1). The AG female, SP female, MP+AG female and MP+SP female were significantly larger (p<0.01) than the control female. The RBV of normal engorged *H. longicornis* female is over 10-fold (Okura *et al.*, 1996). Thus, the effects were clearly induced but incomplete in the present experiments. Copulatory stimuli under experimental conditions also resulted in incomplete or no response in *Dermacentor variabilis* (Papas and Oliver, 1972), *Argas arboresus* (Diehl *et al.*, 1982), *Ornithodoros moubata* and *O. tholozani* (Khalil and Shanbaky, 1975), *Amblyomma americanum* (Oliver *et al.*, 1975), and *A. persicus* (Leahy and Galun, 1972). Incomplete responses are likely induced because the male substances, which are normally transferred into the female genital tract during natural copulation, were injected into the female haemocoel. Whereas, *D. variabilis* females mated with aspermic males engorged normally after receiving spermless spermatophores (Papas and Oliver, 1972). Haemocoelic injection of living sperm or homogenized sperm and homogenized seminal vesicles induced complete oogenesis and oviposition in *O. moubata* (Aeschlimann, 1968; Germand and Aeschlimann, 1977). We think that artificial treatment in the present study could not induce partially effects. It is interesting but a hard problem that stimuli by “haemocoelic” injection of “male germ cells” induced complete reactions only in this experiment. It is known that a few factors influence each other and these actions altered the degree and term of stimulus effects in insects.

Oviposition was observed in females of all groups except for the controls (Table 1). The number of eggs laid (mean±S.E.) in MP female, AG female, SP female, MP+AG female, and MP+SP female were 174.7±23.13, 465.4±219.4, 311.0±45.00, 74.33±9.333, and 136.5±52.72, respectively. A female *H. longicornis* normally oviposits an average of 2552 eggs after pre-oviposition period (5.4-day), and most eggs hatch within about 20 days (Yano *et al.*, 1985). In the experimental groups, the pre-oviposition period was normal but the mean number of eggs laid was 3 to 18% of a naturally mated female. Oogenesis may be proportional to the quantity and quality of nutriments from the blood-meal as in other ticks.

It has also been described that female cuticular plasticization after copulation is induced by haemocoelic injection of synganglion extracts from receptive virgin females, the haemolymph from mated females, and biogenic amines (Okura *et al.*, 1997). This suggests that a possibility for regulation of the neurohormonal system during plasticization. So, in addition, effects on female feeding status after injection with dopamine or the synganglion

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<th>RBV±S.E.</th>
<th>NO±S.E.</th>
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<tr>
<td>Control</td>
<td>1.04±0.16</td>
<td>—</td>
</tr>
<tr>
<td>MP</td>
<td>1.90±1.17</td>
<td>174.67±23.13</td>
</tr>
<tr>
<td>AG</td>
<td>2.88±0.39*</td>
<td>465.40±219.43</td>
</tr>
<tr>
<td>SP</td>
<td>3.45±0.59*</td>
<td>311.00±45.00</td>
</tr>
<tr>
<td>MP+AG</td>
<td>2.77±0.59*</td>
<td>74.33±9.33</td>
</tr>
<tr>
<td>MP+SP</td>
<td>2.58±0.31*</td>
<td>136.50±52.72</td>
</tr>
</tbody>
</table>

Asterisks show significant differences to Control (*p<0.01; **p<0.05). AG, accessory gland extract; MP, mouthparts; NO, number of oviposition; RBV, relative body volume; S.E., standard error; SP, spermatophore extract
extract were also observed (Data not shown). However, significant differences could not confirm.

While, many studies on male factors in about 60 species of insects report their major functions are reduction of receptivity and increase in eggs laid of mated females (reviewed by Eberhard, 1996). It is known that most male substances in insects enter the haemocoel unaltered and stimulate their target organs directly. Although the general rule in insects appears to be direct action, there are also a few insect species such as *Rhodnius prolirus* (Davey, 1985) and *Helicoverpa zea* (Callahan and Cascio, 1963; Kingan et al., 1993a, b; Raina, 1989; Ramaswamy and Cohen, 1992; Snow et al., 1972), in which male seminal substances stimulate the female within her genital tract. Therefore, to obtain a better understanding of tick copulatory stimuli, further observations are needed in regards to existence of mechanisms other than direct action.

In the present study, the number of eggs laid by females in all treatments was much lower than in normal mated females. The engorgement of females was partial even if dopamine, which induces cuticular plasticization in *H. longicornis*, was mixed with the injection materials. It is suggested that cuticular plasticization occurred but feeding activity did not increase. In order to increase the activity, other stimuli such as stimulation via the female genital tract and/or presence of sperm may be needed. These results indicate the existence of more complicated mechanisms in control of female tick reproduction.

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