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The mechanism of oviposition in *Argas (Persicargas) walkerae* (Acari: Argasidae)

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Abstract. The process of oviposition in *A. walkerae* was observed and found to be a sequence of exactly coordinated, interlocking events independent of the age of ticks. Egg-laying always commenced with numerous dorso-ventral movements of the capitulum. The pedipalps were spread during the last dorso-ventral movement and lowered to the ventral body wall embracing the genital aperture on both sides. Simultaneously, the cuticular sac of Gene's organ emerged and immediately everted maximally. Immediately afterwards the vestibulum vaginae prolapsed from the genital aperture touching the cuticular sac. The vestibulum vaginae handed over an egg to the cuticular sac after a brief contact. Then the vestibulum vaginae invaginated, the pedipalps closed and the cuticular sac was retracted. Finally, the capitulum very frequently moved up and down and the free egg was pushed in front of the ticks. The total time of laying an egg averaged 27 min in both 4-week-old and 12-month-old ticks. In 4-week and 12-month-old ticks, egg-laying was preceded by a mean preoviposition period of 6.1 days and 7.5 days, respectively, while the mean oviposition was completed in 14 and 10 days and the average egg totals were 119 and 103.

Key words: Acari, Ixodoidea, *Argas walkerae*, oviposition, mechanism

Introduction

Argas walkerae is widespread in southern Africa, its distribution so far documented includes the Republic of South Africa, Lesotho, Namibia, Zimbabwe and Zambia. This species parasitizes domestic fowls causing considerable economic losses especially as the vector of *Aegyptianella pullorum* and *Borrelia anserina* (Gothe, 1992a, b). In addition, larvae secrete a neurotoxin during feeding, frequently resulting in fatal paralysis (Gothe, 1999).

The multihost feeding pattern characterizes the life cycle of *A. walkerae*. Adults feed and mate several times and females deposit relatively small egg batches, one after each blood meal. The number of gonotrophic cycles, however, varies between individuals and rarely exceeds 8 (Gothe, 1971; Gothe

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and Koop, 1974a, b). This strategy differs from that of ixodid females, which lay several thousand eggs in one continuous cycle and die after oviposition. Consequently, it might be expected that the modus operandi of egg-laying, too, differs between argasids and ixodids.

Egg-laying, however, has been investigated in detail only in ixodid species, namely in *Rhipicephalus evertsi evertsi* (Gothe and Nadler, 1987) and *Dermacentor reticulatus* (Sieberz and Gothe, 2000). The present investigation examines the mechanism of oviposition in *A. walkerae* as well as the sequence and coordination of events, the majority of which were photographically and time recorded. Because more than 90% of engorged females laid eggs and since the preoviposition period was shorter and the egg index (= quotient of the total egg number and the weight of the engorged female) was higher at a temperature near 30°C than of 20°C (Pfeifer, 1990), these studies were conducted at 28°C. To investigate whether the age of the ticks influences oviposition, two groups of females were used, 4 weeks and 12 months after ecdysis.

Materials and Methods

Ticks

Argas walkerae ticks used in the present study were laboratory-reared offspring of ticks collected in a small-holder-type fowl run near Pretoria (Republic of South Africa) in 1986. Chickens were used as hosts for the larvae, nymphs and adults. All host animals were infested only once. Off-host stages were kept at 28°C and 95% relative humidity (R.H.).

Course of oviposition

As a basis for studying the mechanism of egg-laying, the period of preoviposition and oviposition as well as the daily and total number of eggs deposited in relation to the age of female ticks were investigated first. Females engorged and mated after the first blood meal and were used 4 weeks and 12 months after ecdysis. The ticks were put singly in glass tubes which were closed by cotton plugs permeable for water vapour and transferred in an incubator (Memmert, Schwabach, FRG) adjusted to 28°C and 95% R.H. After the start of oviposition, until egg-laying ceased, the ticks were moved daily to new glass tubes and the daily egg output was counted under magnifying glasses. The number of females studied is given in Table 1. As a control, engorged mated females of the same age were left completely undisturbed in the incubator until oviposition ceased. The statistical evaluation was performed by the *t*-test.

Table 1. Average number of eggs daily deposited in 4-week and 12-month-old ticks

Age of ticks	Oviposition day													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
4 weeks ($n = 40$)	7	15	13	15	13	10	11	9	7	6	5	3	3	2
12 months ($n = 38$)	9	11	17	15	13	11	10	8	5	4				

Mechanism of egg-laying

To observe the oviposition process, engorged mated females passing the first gonotrophic cycle were used 4 week and 12 month after ecdysis. The ticks were glued to microscope slides at the posterior ridge of their opisthosoma by means of a double-sided adhesive tape. The immobilized ticks were then taken to a completely darkened room adjusted to 28°C and 40–60% R.H. and were guarded against environmental influences by a plastic dome. Intensity-controlled cold light (Schott, Mainz, FRG) was reflected from a mobile mirror onto the ventral side of the tick. Since adult ticks react phototactically negative (Gothe *et al.*, 1989; Beelitz and Gothe, 1991), ticks were observed at an irradiance of 2.75×10^{-7} mW/cm^2 analogous to crepuscular conditions measured with a precision radiometer (Model IL 1700, International Light, Massachusetts, USA).

Egg-laying was observed under a binocular microscope (Photomakroskop M 400, Wild, Herrsbrugg, Switzerland) with a photographic camera attached (Photoautomat MPS 45, Wild, Herrsbrugg, Switzerland) via the mobile mirror, which was placed on a support approximately 0.5 cm in front of the tick and aimed to view its ventral side. The sequence of events during the laying of one egg and the coordination of the organs involved, as well as their surface structures, were analyzed exactly and photographed and the time taken was manually measured with a stop watch. In the process of laying one egg 11 events were investigated as described in Table 2. In 4-week and 12-month-old females, the modus operandi of oviposition as well as the sequence and coordination of events were examined for 100 and 46 oviposition processes, respectively. For this investigation, females were only used within 3 day after the start of oviposition.

Results

Course of oviposition

At 28°C and 95% R.H., onset of egg-laying was preceded by a mean pre-oviposition period of 6.1 day and 7.5 day in 4-week-old and 12-month-aged

Table 2. Average duration in minutes 'and seconds' (S.D.) of the events 2–10 in the process of laying an egg and average number (S.D.) of dorso-ventral movements of the capitulum before eversion and after retraction of the cuticular sac of Gene's organ (events 1, 11)

Event		Ticks after ecdysis	
		4 weeks	12 months
Event 1:	Number of dorso-ventral movements of the capitulum before eversion of the cuticular sac of Gene's organ	92(48)	84(53)
Event 2:	Duration of one dorso-ventral movement of the capitulum	2″	2″
Event 3:	Total time of dorso-ventral movements before eversion of the cuticular sac	7′38″(4′00″)	7′2″(4′26″)
Event 4:	Time between the last dorso-ventral movement of the capitulum and the maximal eversion of the cuticular sac	1″	1″
Event 5:	Time between the last dorso-ventral movement of the capitulum and the first contact of the cuticular sac with the vestibulum vaginae	<2″	<2″
Event 6:	Time of contact between the cuticular sac and the vestibulum vaginae	<1″	<1″
Event 7:	Time of handing over an egg from the vestibulum vaginae to the cuticular sac	30″(9″)	31″(10″)
Event 8:	Period of egg-embracement by the cuticular sac	9′58″(2′56″)	11′5″(3′33″)
Event 9:	Time of ovipositor eversion	1′30″(17″)	1′19″(10″)
Event 10:	Period of dorso-ventral movements of the capitulum after releasing an egg from the embracement by the cuticular sac	7′35″(3′54″)	7′25″(2′32″)
Event 11:	Number of dorso-ventral movements of the capitulum after releasing an egg from the embracement by the cuticular sac	227(117)	222(76)
Total time of laying an egg		27′17″(5′52″)	27′28″(10′8″)

ticks, respectively. Peak egg output was achieved 2–4 day after the start of egg-laying amounting to 11–17 eggs per day (Table 1). Thereafter, the daily egg output steadily declined. In 4-week and 12-month-old females, the mean oviposition period was completed in 14 day and 10 day and the average egg totals were 119 and 103, respectively (Table 1).

Four week ($n = 39$) and 12-month-old females ($n = 38$) left undisturbed during the oviposition deposited on average 142 and 113 eggs, respectively.

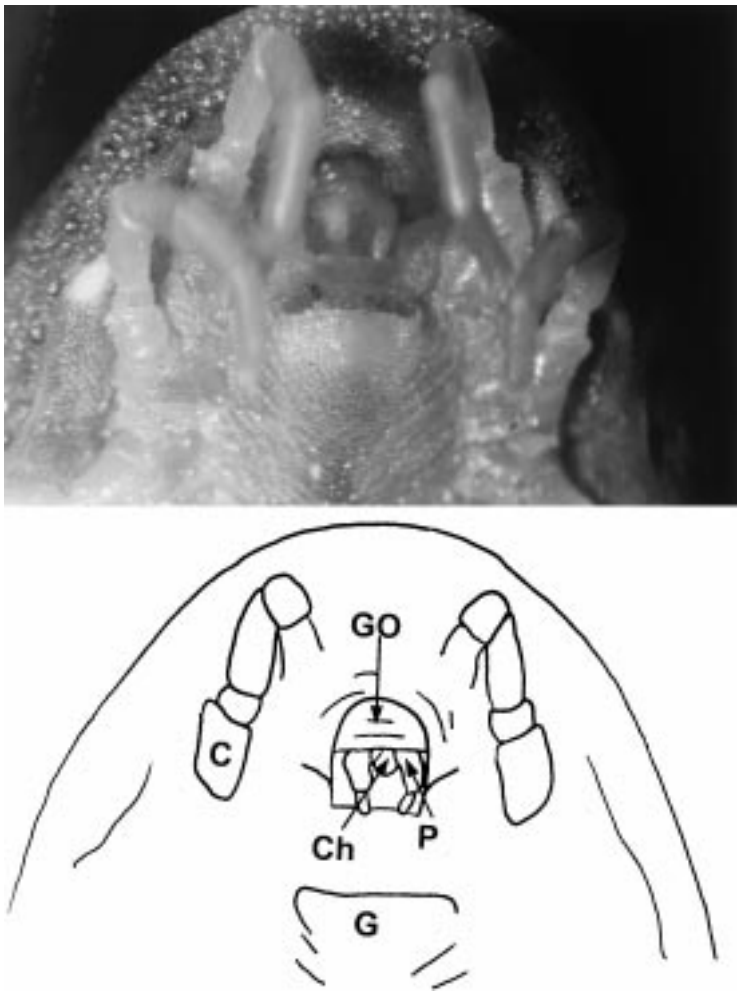

Figure 1. Lowered capitulum: coxa I (C); chelicerae (Ch); genital aperture (G); cuticular sac of Gene's organ (GO); pedipalps (P).

The statistical evaluation revealed no significant differences between females left undisturbed or checked daily during egg-laying, but 12-month-old females laid significantly less eggs than 4-week-aged ticks.

Mechanism of egg-laying

The sequence of events in the course of laying an egg was correspondent in both the 4-week and 12-month-old ticks. However, independent of the age, the number and the total time of dorso-ventral movements of the capitulum before eversion and after retraction of the cuticular sac of Gene's

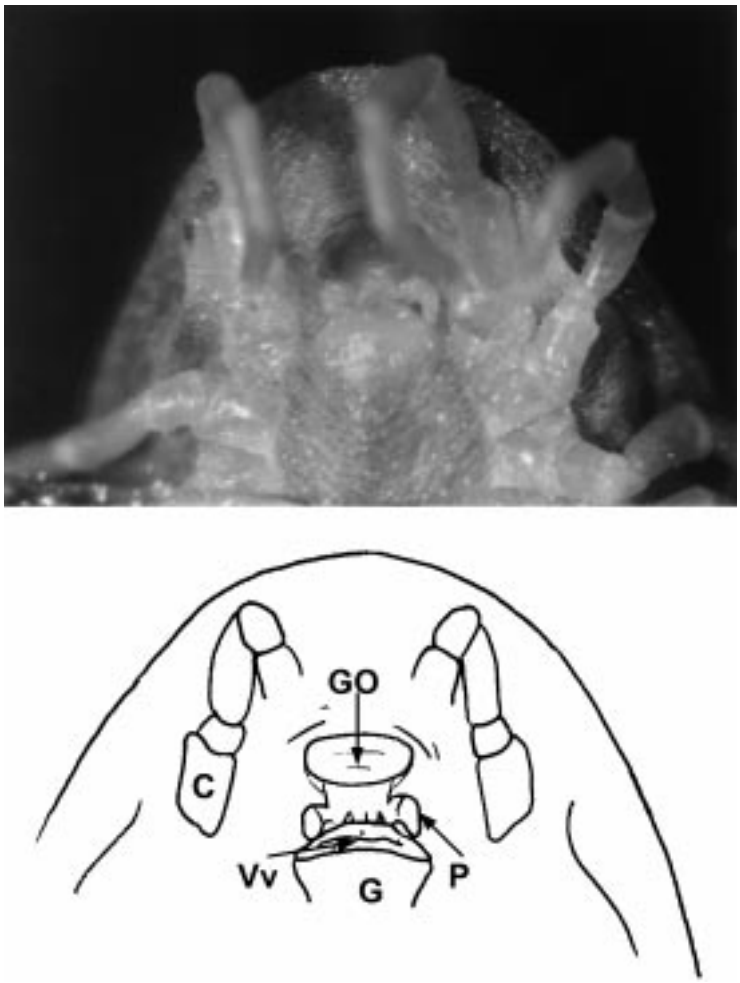

Figure 2. Spread pedipalps lowered to the ventral body wall embracing the genital aperture on both sides: coxa I (C); genital aperture (G); cuticular sac of Gene's organ (GO); pedipalps (P); vestibulum vaginae (Vv).

organ, as well as the period of egg-embracement by the cuticular sac, varied considerably (Table 2).

Egg-laying always commenced with numerous dorso-ventral movements of the capitulum (Figure 1). With time, these movements became more rapid and extended more deeply towards the genital aperture until this opening was touched. The pedipalps remained closed, but were spread during the last dorso-ventral movement of the capitulum and lowered to the ventral body wall embracing the genital aperture on both sides (Figure 2). Within 1–2 s after the last movement of the capitulum, the glittering, grey-white cutic-

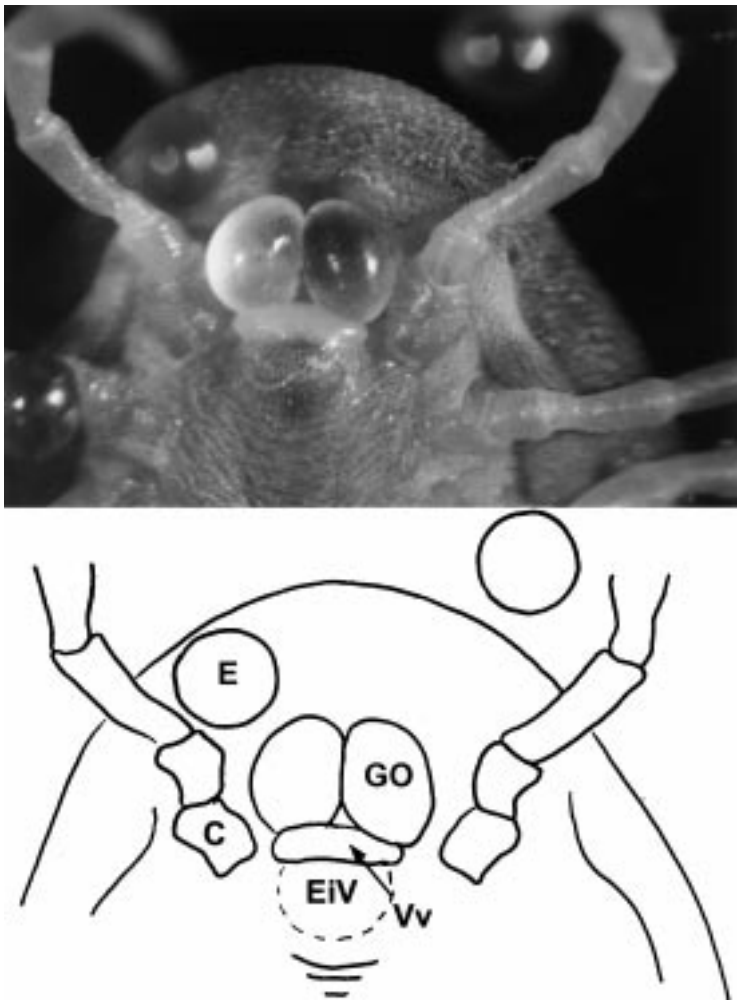

Figure 3. Contact between the maximally everted cuticular sac of Gene's organ and the prolapsed vestibulum vaginae: coxa I (C); egg (E); egg in vestibulum vaginae (EiV); cuticular sac of Gene's organ (GO); prolapsed vestibulum vaginae (Vv).

ular sac of Gene's organ with its two horns emerged at the camerostomal aperture and immediately everted maximally (Figure 3). Simultaneously, the vestibulum vaginae prolapsed from the genital aperture forming an extended tube and touching both horns of the cuticular sac (Figure 3). The vestibulum vaginae operated as an ovipositor and handed over an egg to the two horns of the cuticular sac after a brief, but intensive, contact (Figure 4). The egg was embraced by the horns of the cuticular sac, except for a small sector (Figure 5). After handing over an egg, the vestibulum vaginae invaginated immediately (Figure 6).

Figure 4. Handing over an egg from the vestibulum vaginae to the cuticular sac of Gene's organ: coxa I (C); egg (E); everted cuticular sac of Gene's organ (GO); prolapsed vestibulum vaginae (Vv).

After the retraction of the ovipositor the pedipalps closed and the dorso-ventral movements of the capitulum started again while the egg was turned by the horns of the cuticular sac (Figure 7). Due to the retraction of the cuticular sac, the egg became free (Figure 8). The pedipalps remained closed and the dorso-ventral movements continued and their amplitude upwards and downwards continuously decreased with time until the capitulum finally remained in an elongated position along the longitudinal axis of the tick body. The total

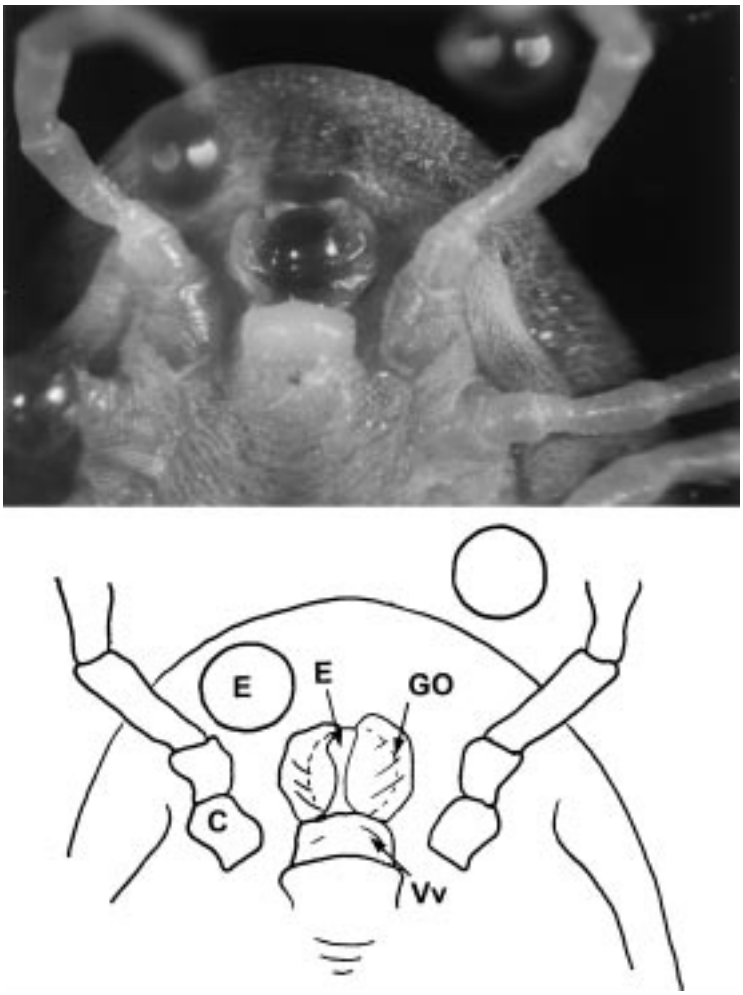

Figure 5. Embracement of an egg by the two horns of the cuticular sac of Gene's organ, except for a small sector: coxa I (C); egg (E); everted cuticular sac of Gene's organ (GO).

time of laying an egg, covering the events 2–10, averaged 27 min both in 4-week-old and 12-month-aged ticks (Table 2).

Discussion

Oviposition in *A. walkerae* is a very delicate process prone to disturbances. However, under the experimental design used in this study, females which commenced to lay an egg always finished this process. The sequence, the coordination and the mean periods of the events occurring in the course of an

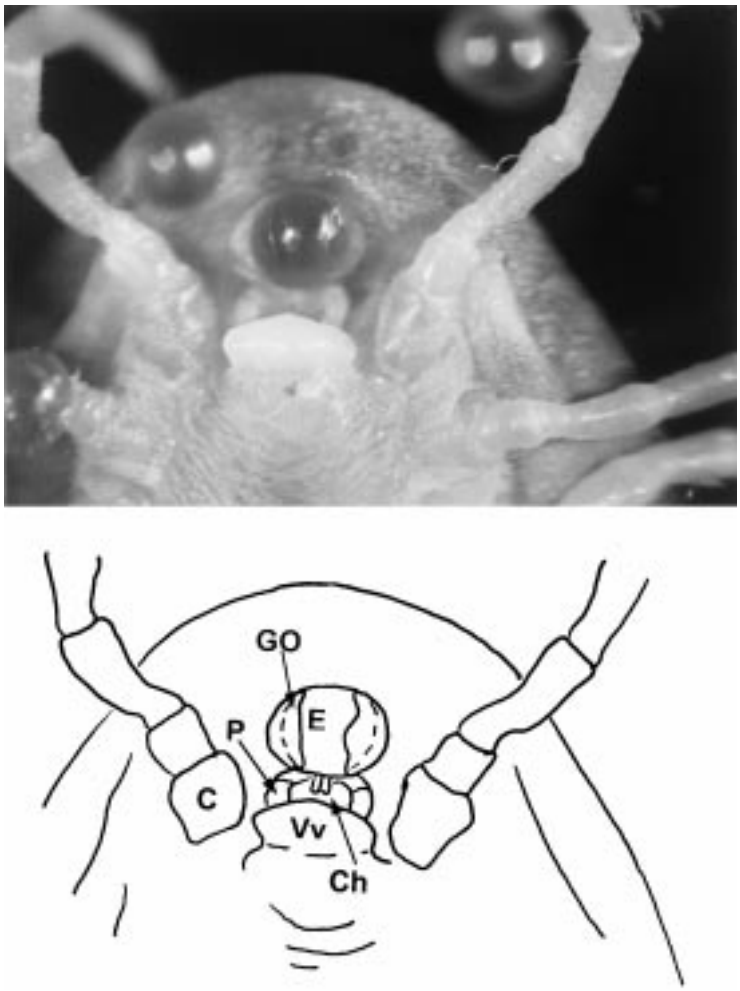

Figure 6. Partial retraction of the vestibulum vaginae into the genital aperture: coxa I (C); chelicerae (Ch); egg (E); cuticular sac of Gene's organ (GO); pedipalps (P); vestibulum vaginae (Vv).

oviposition process as well as the number of dorso-ventral movements of the capitulum before eversion and after retraction of the cuticular sac of Gene's organ did not differ between 4-week and 12-month-old ticks, indicating that the age of ticks does not influence the modus operandi of oviposition.

A significant correlation of the sequence of events during oviposition in *A. walkerae* and the egg-laying process in other argasid species cannot be drawn, since previous reports concerning *A. arboreus* (Hafez *et al*., 1972), *Ornithodoros moubata* (Brumpt, 1910; Lees and Beament, 1948) and *O. savignyi* (Brumpt, 1910) only describe egg-laying in few sentences without analysis

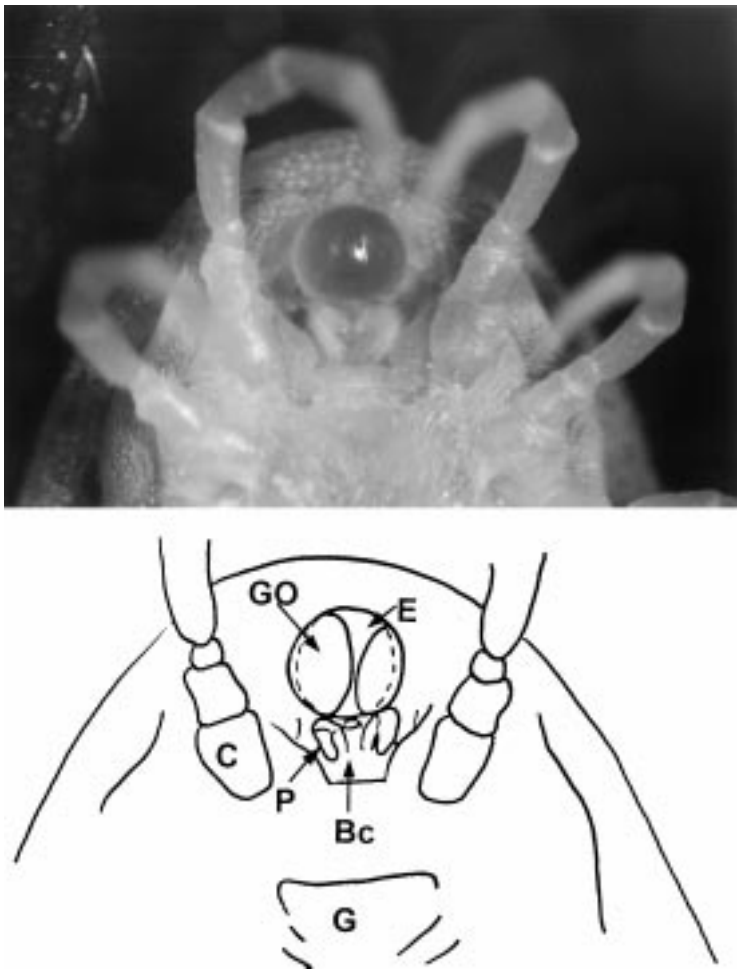

Figure 7. The horns of the cuticular sac turning an egg: basis capituli (Bc); coxa I (C); egg (E); genital aperture (G); cuticular sac of Gene's organ (GO); pedipalps (P).

and photographic documentation of the events. Compared with ixodid species, investigated in detail in *R. evertsi evertsi* (Gothe and Nadler, 1987) and *D. reticulatus* (Sieberz and Gothe, 2000), the modus operandi of oviposition completely differs in several events from *A. walkerae*. In contrast to the argasid species, females of these ixodid species always commenced egg-laying with only one, but very jerky lowering of the capitulum while the pedipalps were spread and lowered to the ventral body wall embracing the genital aperture on both sides. In further contrast to *A. walkerae*, the cuticular sac of Gene's organ in the ixodid ticks was pushed out and retracted several times and its eversion increased continuously touching initially the

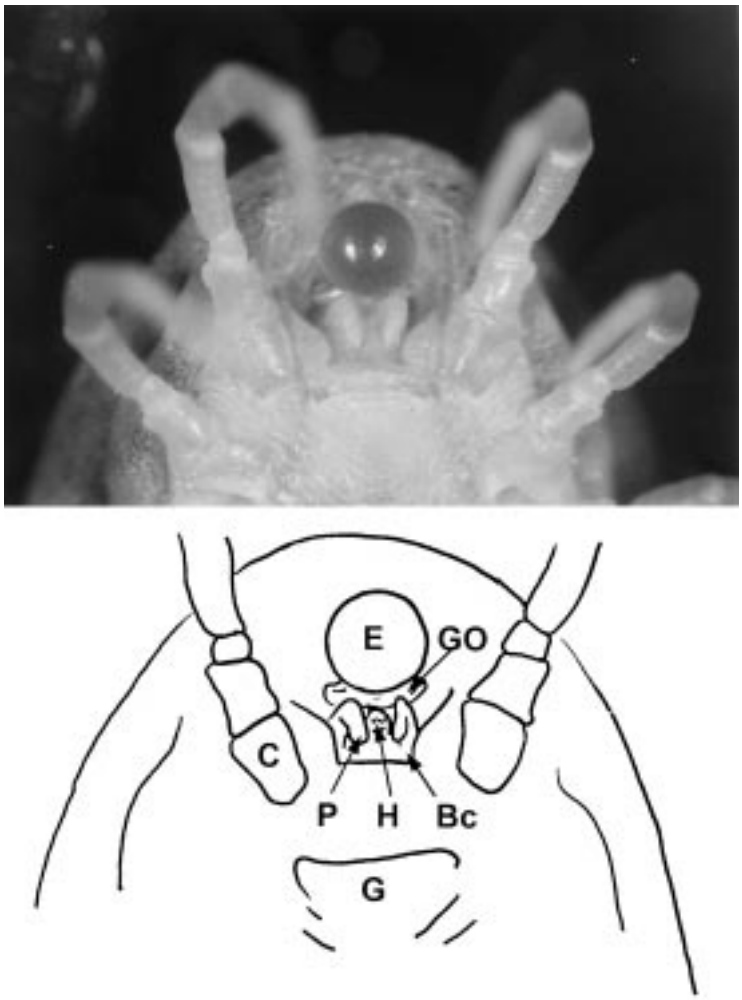

Figure 8. Deeply retracted cuticular sac of Gene's organ: basis capituli (Bc); coxa I (C); egg (E); genital aperture (G); cuticular sac of Gene's organ (GO); hypostome (H); closed pedipalps (P).

porose areas only, then also the chelicerae and the insides of the pedipalps, to finally rise above the pedipalps. Further distinct differences exist with regard to the period of the egg-embracement by the cuticular sac of Gene's organ, which averaged 67 s in the first and 200 s in the last third of oviposition in *D. reticulatus*, but amounted to a mean of 10–11 min in *A. walkerae*. In addition, unlike *A. walkerae*, after retraction of the ovipositor the pedipalps of the ixodid females carried out circular movements which were accompanied by up-and-down motions of the chelicerae while the egg was transported onto

the dorsal area of the tick by means of one vigorous rising of the capitulum. In contrast to that, after retraction of the vestibulum vaginae and the cuticular sac the capitulum very frequently moved up and down for approximately 7 min in *A. walkerae* and the free egg was pushed in front of the tick.

The peculiar and always numerous occurring dorso-ventral movements of the capitulum before eversion and after retraction of the cuticular sac of Gene's organ are difficult to explain. It may be speculated that the capitulum movements before eversion of the cuticular sac cause numerous increases in the hemolymph pressure which are necessary to squeeze sufficient secretion of the glands associated with Gene's organ from the lumen between the epithelial and the cuticular sac into the cuticular sac. The numerous capitular movements after retraction of the cuticular sac by means of muscles, however, may indicate that the cuticular sac is physiologically placed within Gene's organ only by the frequent change in hemolymph pressure.

Since depositing one egg required 27 min, it is justified to conclude that very long rests are taken between the completion of the previous and the start of the subsequent oviposition process. Indeed, only 35–37 eggs were laid within 3 days after the start of oviposition, whereas 160 eggs would have to be expected if females would continuously deposit eggs every 27 min. Because ticks were observed in this study which laid several eggs without a break or deposited eggs at intervals of many hours, the periods between oviposition processes have to be considered as variable. It is further justified to conclude that the intervals steadily increase during the course of oviposition, since the daily egg output continuously decreased to 2–4 eggs at the last day of oviposition.

As previously noted (Sieberz and Gothe, 2000), embracement and impregnation of eggs by the cuticular sac of Gene's organ are indispensable. In other tick species, for example *O. moubata* and *Ixodes ricinus* (Lees and Beament, 1948), *Haemaphysalis longicornis* (Kakuda et al., 1992) and *D. reticulatus* (Sieberz and Gothe, 2000) it has been demonstrated that after blockage of Gene's organ, eggs dried up quickly after deposit, shrivelled and were not viable, or only a minor quota. The chemical nature of the secretion of Gene's organ, however, is still unknown.

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