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MORPHOLOGICAL OBSERVATIONS ON THE FORMATION OF THE SPIRACLE OF ARGAS PERSICUS (OKEN) (ACARINA : METASTIGMATA : ARGASIDAE) DURING MOULTING

BY P. J. A. PUGH 1,2, P. E. KING 1 & M. R. FORDY 1

ARGAS PERSICUS
NYPH		ARGAS PERSICUS
SPIRACLE
MORPHOLOGY
ABSTRACT : The morphology of the nymphal spiracle of Argas persicus (Oken) is described in an advanced developmental state during the moult. This modification of the "simple type" of tracheal/spiracular moulting is compared with the "elateroid type" of the Ixodidae.

ARGAS PERSICUS
NYPH		RÉSUMÉ : La morphologie du spiraculum de la nymphe d’Argas persicus (Oken) est décrite dans un état avancé du développement, au cours de la mue. Cette modification d’un « type simple » de mue trachéenne/spiraculaire est comparée au type elateroïde des Ixodidae.

INTRODUCTION

The Argasidae or soft ticks, are multi-host, blood-feeding ectoparasites, which occur in the nests of birds and shelters of small mammals throughout temperate and tropical regions (COOLEY & KOHLS, 1944 ; EVANS et al., 1961 ; KRANTZ, 1978 ; TILL & GRAY, 1989). Like other arthropods, argasid ticks have a cuticular external covering and lining to the foregut, hindgut, ducts of the dermal glands and the tracheal system (HACKMAN & FILSHIE, 1982), and require a series of three or more moults during development (HOOGSTRAAL et al., 1975 ; ROBERTSON et al., 1975 ; HACKMAN & FILSHIE, 1982 ; ROSHDY et al., 1982).

Moult begins with apolysis, during which most of the old endocuticle is resorbed and ends with ecdysis, when the exuvial epi- and exo-cuticle are discarded (JENKINS & HINTON, 1966 ; ROSHDY, 1974).

Larval Ixodidae lack tracheae (BALASHOV, 1968 ; BIDYASAR & MISTA, 1974), though a "respiratory system" has been described opening between coxae I and II in some Argasidae, namely in Argas and Ornithodorus species (OSTROUMOVA, 1939 ; SCHULZE, 1949 ; THEODOR & COSTA, 1960 ; BALASHOV, 1968 ; ROSHDY et al., 1982). All post-larval stages possess a tracheal system comprising a single pair of spiracles or stigmata, connected to a system of branched tracheae and terminating in intracellular

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The extensive literature on the intermoult spiracle morphology of the Ixodidae or hard ticks has been reviewed by Pugh et al., (1988, 1990), but there are few studies on the Argasidae (Robinson & Davidson, 1913; Mellanby, 1935; Browning, 1956; Sixl et al., 1971; Woolley, 1972; Bidyasar & Misra, 1974). In Argas persicus (Oken), the miana bug or fowl tick, which is an ectoparasite of birds (Krantz, 1978; Till & Gray, 1989), the imperforate macula is separated from a crescent shaped, porose spiracular plate by a narrow, open ostium. The spiracular plate comprises a sieveplate, labyrinth and baseplate. The thin sieveplate is perforated by aeropyles and overlies the labyrinth consisting of an airspace containing numerous elongate pedicels, which extend from the sieveplate to the solid cuticular baseplate. Beneath the macula are two chambers, namely a sub-ostial space and the tracheal atrium which connect the labyrinth airspace and main tracheal trunks. These are partially separated by a projection resembling the ixodid atrial valve in its position (Pugh et al., 1991).

The formation of the tracheal and spiracular intima prior to moulting has been described in both the Hexapoda (Jenkins & Hinton, 1966; Chapman, 1982) and the Ixodidae (Roshdy, 1974). The present study examines the morphology of the spiracle in specimens of A. persicus, at a stage of moulting where the spatial relationship between the new and exuvial systems may be determined.

**Material and methods**

Alcohol fixed, specimens of A. persicus were obtained from a museum collection, from which inter-nymphal moult specimens in late apolysis, i.e. still surrounded by their ecdysial cuticles were selected for study. These were rinsed in fresh ethanol, rehydrated to distilled water and transferred to glycerol for cleaning in an ultrasonic bath, using “Micro” detergent (International Products Corporation, Trenton, New Jersey, U.S.A.), then dehydrated in an acetone series, dried in air and mounted on aluminium stubs, using quick setting epoxy resin and colloidal silver. The specimens were then coated with gold in a Polaron Mark II sputter coating unit and examined in a JEOL 35C scanning electron microscope. Some specimens were subsequently removed from their stubs, cut in various planes with a razor blade, remounted and recoated as described above. The exuvial linings of other specimens were removed, mounted on stubs and coated to reveal further details.

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**Fig. 1-9: Argas persicus (Oken).** Scanning electron micrographs of spiracle in advanced apolysis.

1 & 2. — Different aspects of a section through a spiracle, showing the exuvial macula (m) and sieveplate (P) forced together, sealing the new ostium, bordered by the surrounding new macula (M) and sieveplate (P). The membrane (n) covers the new sieveplate. Both scales : 20 µm.

3 & 4. — Complementary sections of spiracle in Figs. 1 & 2, showing the invaginate new macula (M) below its ecdysial counterpart (m). The new (P) and ecdysial (P) sieveplates are separated by a membrane (n), the ecdysial spiracle protrudes beyond the new ostium, which is greatly dilated. The sub-ostial space is empty, but the ostium and tracheal (A) are plugged by the ecdysial lining (a). The ecdysial tracheal lining (t) is visible within the new tracheal lining (T) Scales : 20 & 50 µm respectively.

5. — Spiracular plate in which the ecdysial lining has been removed, showing the enlarged ostium (O) and macula (M) invaginated into the spiracular wall opposite the sieveplate (P) (c.f. Fig. 6). Scale : 20 µm.

6. — Immmoult spiracle soon after ecdysis, showing the sieveplate (P), macula (M) sealing off the ostium (O) (c.f. Fig. 5). Scale : 20 µm.

7. — Surface of new sieveplate, showing the aeropyles (e) with distinct raised rims and with the position of the underlying pedicels revealed by the protruberances (b). Scale : 2 µm. 8 & 9. — Ecdysial tracheal lining removed from the new spiracle in Fig. 5, including the sub-ostial space (s), tracheal atrium (a) attached to the ecdysial cuticle (c). Both scales : 20 µm.
Observations and Discussion (Figs. 1-10)

During moulting, the lining of the new spiracle is formed around the ecdysial one, with the sieveplate, labyrinth and baseplate formed as one (spiracular plate) unit and the macula as another, as in the Ixodidae (RosHDy, 1974). The position of the new spiracular plate forces the exuvial one into the ostium, the two being separated by a membrane, which may be part of the ecdysial membrane, and which protects the aeropyles of the new sieveplate from moulding fluid and/or blockage by cellular debris. The surface of the new sieveplate is not indented as in post-moulting specimens (PUGH et al., 1991), but is ornamented with small protruberances, corresponding to the tops of the underlying pedicels, while the rims of the aeropyles are slightly raised. Initially, the pedicels are thin with central canals which aid flexibility during ecdysis, but after moulting they become thicker and their canals are occluded. Differences in morphology between newly formed and intermoulting spiracular plates suggest that their formation continues after moulting by the passage of material onto the surface via the pedicel canals which become blocked when the process is complete.

The two components of the new spiracle are not formed in situ in their post-moulting positions, but are subsequently moved into position by haemolymph pressure. During ecdysis, the new sieveplate moves upwards from the wall of the sub-ostial space, while the new macula is everted from the haemocoel opposite the sieveplate to block the dilated ostium when the temporary plug, formed by the ecdysial macula and spiracular plate, is removed. Withdrawal of the exuvial tracheal system and spiracle is aided by moulding fluid which digests the endocuticular moiety of the tracheal intima (HINTON, 1947) and by the inward collapse of the main ecdysial tracheal trunks into the dilated new sub-ostial space. This also allows air into the new tracheal ducts during ecdysis. When moulting, ticks may reduce transpiration by means of a number of mechanisms, including restricting moulding to humid micro-environments (RosHDy, 1974; KNUlle & Rudolph, 1982). These responses also reduce the tick's oxygen requirements, which may be important because the airways are partially obstructed by the exuvial system.

HINTON (1947) suggested that there are three methods of moulding the spiracular and tracheal lining in insects: (a) Simple, in which the lining is withdrawn through an unguarded respiratory opening. This is impossible in spiracles guarded by protective devices which therefore require one of two specialised types of moulding, namely (b) Panorpoid, where the ecdysial lining is pulled out through a central opening, which is subsequently sealed by the contraction and hardening of the surrounding cuticle, or (c) Elateroid, in which the tracheal lining is pulled through an ecdysial tube formed to one side of the respiratory opening and sealed at the completion of ecdysis.

Based upon RosHDy & HeFNawY's (1973) suggestion that the ixodid ostium can open after moulding, forming a passage for both air and removal of the exuvial tracheal system, a simple moulding pattern has been inferred for the ixodid tracheal intima (RosHDy, 1974). However, it has been shown that the ostium cannot open after moulding (HINTON, 1967; PUGH et al., 1988, 1990), leaving the sieveplate aeropyles as the only functional openings. This supports HINTON’s (1967) suggestion that the Ixodidae have an elateroid system, where the exuvial tracheae are withdrawn through the ostium, which subsequently collapses to form an ecdysial tube.

The Argasidae, like the Ixodidae also withdrawn the ecdysial tracheal system through the new ostium, but unlike that of the Ixodidae, the argasid ostium remains open and is also the route for inspired air (PUGH et al., 1991), giving it a dual function as (1) a controllable intermoulting respiratory orifice, when the macula is everted into the ostium and (2) an ecdysial tube during moulting, when the macula is inverted into the ostial wall.

Thus although the mechanism of spiracular moulding in the Argasidae is superficially similar to that described in the Ixodidae by HINTON (1967), it is by definition a modification of the simple type because the tracheae are withdrawn via functional respira-
Fig. 10: *Argas persicus* (Oken). Diagram of spiracle in an advanced state of development during the moult (based on Figs. 1-4). This shows the relationship between the and ecdysial spiracles and main tracheal trunks. The crumpled ecdysial tracheal system is contiguous with the ecdysial exoskeleton and occupies the dilated lumen of its new counterpart. The ecdysial sieveplate and macula form a plug occluding much of the new ostium, which along with the new sub-ostial space are very dilated and the new macula infolded into the wall of the ostium to accommodate the partly collapsed ecdysial intima. New and ecdysial sieveplates are separated by a membrane.

Key to lettering:

- **spiral**
  - New ecdysial

- **A** : tracheal atrium
- **C** : cuticle
- **M** : macula
- **n** : (ecdysial?) membrane separating sieveplates
- **O** : ostium
- **P** : sieveplate
- **R** : cuticular ridges (below atrial valve)
- **S** : sub-ostial space
- **V** : atrial valve

SUMMARY

During moulting, the ecdysial tracheal/spiracular lining is moulted into the lumen of the new one, all new parts being in direct contact with the corresponding ecdysial ones, except for the new sieveplate/labyrinth/baseplate which is produced as a single spiracular plate unit below the exuvial baseplate and covered by a thin membrane. This moves upwards to lie opposite the new macula which is formed below its exuvial counterpart on the opposite side of the dilated ostium. The exuvial tracheal intima is withdrawn in one piece through the ostium, which then closes when the new macula everts, reducing transpiration. Both the displacement of the spiracular plate and eversion of the macula are a result of increases in haemolymph pressure.

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