Insectes soc. 48 (2001) 63–66
0020-1812/01/010063-04 $ 1.50+0.20/0

Insectes Sociaux

Research article

Functional morphology of the metapleural gland in the leaf-cutting ant *Acromyrmex octospinosus*

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Received 7 August 2000; revised 6 December 2000; accepted 13 December 2000.

Summary. The metapleural gland is unique for ants and its main function is the production of antibiotics. The gland is considered to be of particular significance in leaf-cutting ants, which need to protect both themselves and their clonally reproducing fungal symbiont from pathogens and microbial competitors. In a detailed study of the histology of the metapleural gland of the leaf-cutting ant *Acromyrmex octospinosus* the diameter of the storage room (bulla) is shown to be highly positively correlated (r = 0.84) with the number of secretory cells inside the gland. This justifies the use of the easily obtainable bulla diameter as an approximation of the size of the actual metapleural gland in e.g. studies of gland allometry. Histological cross sections also show that contraction of the rather massive thoracic muscles may lead to compression of the collection chamber of the metapleural gland and may thus allow active enhancement of the flow of gland secretion to the reservoir behind the bulla.

Key words: Metapleural gland, *Acromyrmex octospinosus*, antibiotics, allometry, morphology.

Introduction

The exocrine metapleural gland is unique for ants. Positioned pair-wise at the rear end of the mesosoma, it produces antibiotic substances that flow out over the cuticle, providing protection against a series of microbial and fungal pathogens (Maschwitz et al., 1970, Schildknecht and Koob, 1971; Maschwitz, 1974; Beattie et al., 1985, 1986; Veal et al., 1992; Nascimento et al., 1996; A.N.M. Bot, D. Ortius-Lechner, K. Finster, R. Maile and J.J. Boomsma, in prep.). In leaf-cutting ants these secretions may be even more important than in other ants, as they have been hypothesised to also control pathogens and competing micro-organisms in the fungus garden (North et al., 1997).

One aspect that is likely to be correlated with the functional significance of the metapleural gland is its relative size. Earlier work has shown that metapleural glands of leaf-cutting ant workers are relatively large and that this is particularly so in the smallest size class of workers of *Atta* (Wilson, 1980) and *Acromyrmex* (Bot and Boomsma, 1996). This suggests that allocation of resources to metapleural gland secretions is most important in the minor workers, which predominantly tend the fungus and care for the brood. However, allometry studies have relied exclusively on external measures of the bulla, the visible cover of the storage reservoir of the metapleural gland, whereas the exact relationship between the bulla and the size of the internal gland has remained unknown. Earlier work on *Atta* by Schoeters and Billen (1993) has led to estimates of about 400 gland cells in medium workers, soldiers and queens, similar to an earlier estimate of 473 gland cells in the metapleural gland of a medium worker of *Atta sexdens* by Hölldobler and Engel-Siegel (1984). Minor workers have relatively well developed metapleural glands both internally and externally, but the size of the internal gland of minors has never been quantified and related to gland sizes in larger workers.

Material and methods

In this study we use histological techniques to describe the structure of the metapleural gland of the leaf-cutting ant *Acromyrmex octospinosus* and to correlate the size of the internal part of the metapleural gland with the diameter of the bulla and the overall body size parameters pro-

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notum width and head width. Because of the labour intensiveness of the used techniques the total number of investigated workers was limited to six (two majors, two minors and two of intermediate size). All were taken from *A. octospinosus* colony # 19, which was collected in 1994 in Gamboa, Panama and which had been kept in the laboratory since. The legs and heads were removed from the ants after which measurements of the width of pronotum and head and of the diameter of the bulla were taken with a Lasico digital filar eye piece. Directly after these measurements the mesosomas were fixed with Bouin fixative. After one day they were cut longitudinally in two halves to enhance penetration of the fixative to the tissues, and after one more day they were transferred to 75% ethanol which was renewed several times to wash away the fixative. The tissues were subsequently dehydrated in ethanol solutions of increasing concentration (75%–99.6%) and were finally embedded in Spurr resin. Microtome cross sections were 6 µm thick for large and medium workers and 4.5 µm thick for minor workers. Sections were stained with Methylene Blue and Azure II (Sigma) and examined with light microscopy. Microscopic measurements of the size of the cluster of glandular cells were taken with a 0.01 mm graticule (Leica). For all measurements we used the largest possible magnification of the cell cluster. The data for the depth (ipsilateral to lateral) of the glandular cluster are based on the number of slices on which the gland was visible multiplied by the thickness of the sections. Anterior-posterior, dorsal-ventral and ipsilateral-lateral measurements were subsequently averaged for each worker. Gland cell counts were made with camera lucida microscope drawings on translucent paper.

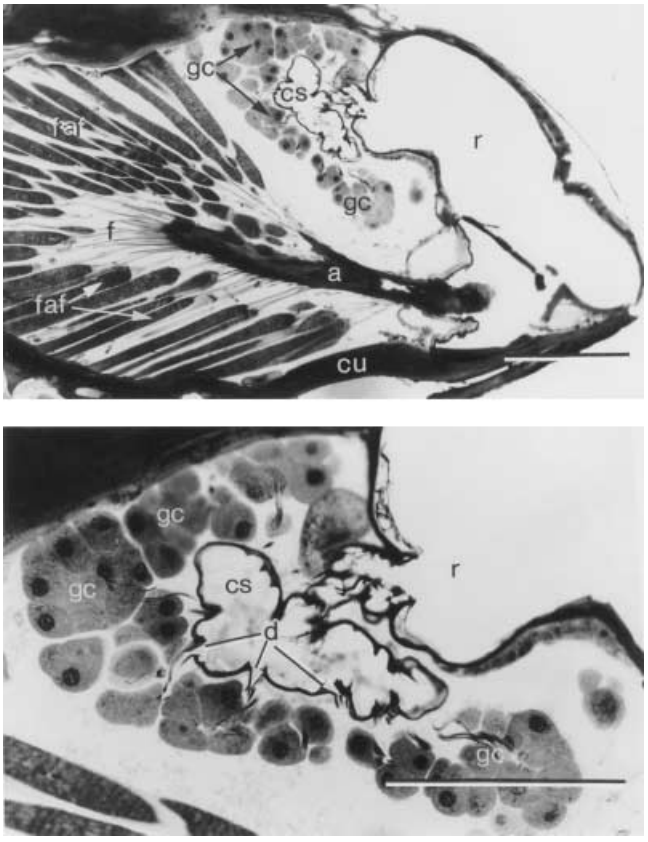

Figure 1. The structure of the metapleural gland of *Acromyrmex octospinosus*. Scale bars are 200 µm long. Cu = cuticle, gc = gland cells, d = ducts, cs = collecting sac, r = reservoir, faf = filament attached fibers, f = filament, a = apodeme

Results and discussion

The gland cells in attine metapleural glands form clusters which are connected to a collecting chamber by ducts and from which the secretion flows out into the reservoir behind the bulla (Hölldobler and Engel-Siegel, 1984; Schoeters and Billen, 1993). Our sections (Fig. 1) confirm this and show that the metapleural gland structure in *Acromyrmex octospinosus* is similar to that found in other ants (but see below). The diameter of the bulla is highly positively correlated ($r = 0.84$) with the size of the internal gland (Fig. 2). The slope of the log-log relationship between bulla size and internal gland size is 0.78 (Fig. 2), which means that the internal gland size increases less than proportional with the diameter of the bulla. Major workers were already known to have relatively small bullas compared to their body size (Wilson, 1980; Bot and Boomsma, 1996), but the difference between small and large workers in the internal metapleural gland structure relative to body size appears to be even more striking. The slopes of the correlations between internal gland size and pronotum width and head witdth are only 0.33 and 0.35, respectively (Fig. 2). Cell counts that correspond to the three size classes of workers that were used for this study were estimated to be between 200 and 300 for small workers, between 350–450 for medium sized workers and between 500 and 600 for large workers. Also these estimates are positively correlated with the size of the bulla (Fig. 3).

The positive correlation between the bulla size and the size of the internal metapleural gland justifies the use of the bulla diameter as an approximation of the size of the actual metapleural gland (Wilson, 1980; Bot and Boomsma, 1996). A larger gland size will likely imply a larger capacity for the production of antibiotic substances, even though this capacity may not be used to its full extent at all times. A reduction of gland productivity may occur under stressful circumstances or with old age (Maschwitz et al., 1970; D. Ortius, pers. comm.; A.N.M. Bot, pers. obs.). A high metapleural gland productivity will most likely result in a better protection of the individual ant and its surroundings, because at least some of the compounds are volatile and will therefore disappear from the environment if they are not produced continuously (Maile et al., 1998).

There is an additional potentially important aspect which previously has not been noticed. This concerns the way in which secretion is transported from the collection chamber to the reservoir behind the bulla. This transport has been assumed to be entirely passive, and the thoracic muscles, labelled 103 and 105 in *Formica polyctena* by Markl (1966), were suggested to be involved in coxa movement only. Our current sections show that contraction of these rather massive muscles (called filament attached fibers in Fig. 1) may also lead to compression of the collection chamber of the metapleural gland and may thus enhance the flow of chemical substances to the reservior behind the bulla. However, it is unlikely that the highly sclerotized bulla is also compressed by contractions of these thoracic muscles. The bulla opens just above the coxa of the hind legs, so that capillary trans-

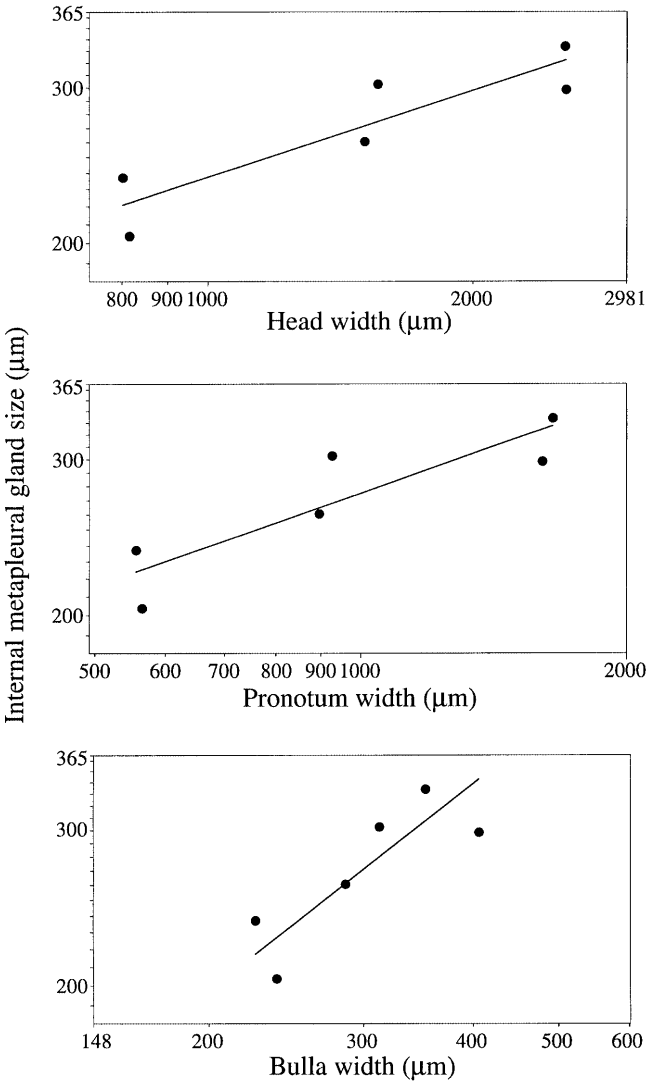

Figure 2. Power functions of the size of the internal metapleural gland (average of ipsilateral-lateral, dorsal-ventral and horizontal measurements) and a) worker head width ($Y = 24.8X^{0.33}$; r = 0.90), b) worker pronotum width ($Y = 25.0X^{0.35}$; r = 0.89), c) worker bulla width ($Y = 3.2X^{0.78}$; r = 0.84). Data on both axes were log-transformed prior to calculating the correlations. The lines given in the figures are first principal component axes and not regression lines

port of secretions from the reservior behind the bulla to the epicuticle is likely to be passive as was inferred previously for *Atta* by Schoeters and Billen (1993).

The high degree of similarity in metapleural gland structure between *Atta* and *Acromyrmex* species suggests that the functional significance of the metapleural gland for defence against pathogenic micro-organisms has not changed significantly with the evolutionary transition to the extremely large mature colony sizes that are typically found in *Atta* species. However, even though colonies of *Acromyrmex* are smaller than those of *Atta*, they can contain several tens of thousands of workers and are consequently large compared to colonies of the lower attines, many of which contain fewer than 100

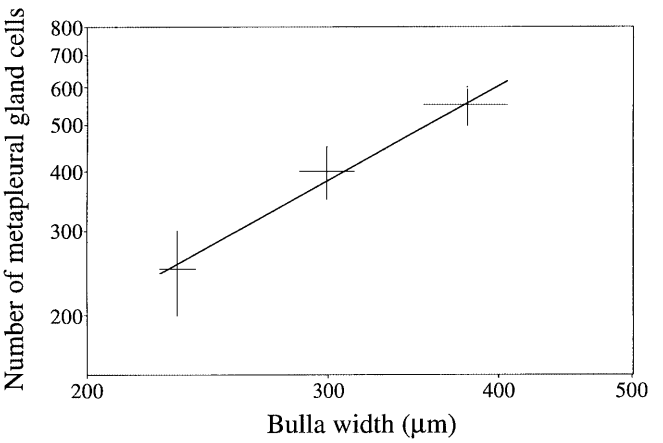

Figure 3. Correlation between the estimated ranges of gland cell numbers for each of the worker size classes and the size of the bulla. Both axes are logarithmic and the power equation is: $Y = 0.046X^{1.6}$

individuals (see e.g. Villesen et al., 1999). A comparative study of the metapleural gland morphology should thus include lower attines to allow any firm conclusions about the function of the metapleural gland in relation to colony size.

Acknowledgements

We thank the Smithsonian Tropical Research Institute for providing logistic help and facilities to work in Gamboa, and the Instituto Nacional de Recursos Naturales Renovables (INRENARE) for permission to sample ants in Panama. The work of A.N.M. Bot was funded by an EU-Marie Curie (B20) fellowship. The stay of A.N.M. Bot at the University of Würzburg was facilitated by the EU-TMR network on 'Social Evolution' of the Universities of Aarhus, Copenhagen, Firenze, Keele, Sheffield, Uppsala, Würzburg and the ETH Zürich, financed by the Eurpean Commission via the Training and Mobility of Researchers (TMR) programme.

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