Epidermal Glands in the Abdomen of a Basal Ant *Dinoponera lucida* (Formicidae: Ponerinae)

JOSÉ EDUARDO SERRÃO,[1]* RAFAEL CUNHA A. CASTRO,[1] JOSÉ COLA ZANUNCIO,[2] CLÉA SANTOS FERREIRA MARIANO,[3] AND JACQUES HUBERT CHARLES DELABIE[3]
[1]*Departamento de Biologia Geral, Universidade Federal de Viçosa, 36570-000 Viçosa, Minas Gerais, Brazil*
[2]*Departamento de Biologia Animal, Universidade Federal de Viçosa, 36570-000 Viçosa, Minas Gerais, Brazil*
[3]*UPA, Laboratório de Mirmecologia, Convênio UESC-CEPEC, 45600-000 Itabuna, Bahia, Brazil*

KEY WORDS Hymenoptera; cuticular hydrocarbons; oenocytes; insect; zoology

ABSTRACT The basal ant *Dinoponera* (Hymenoptera: Ponerinae) has lost the morphologic queen caste so that all females may be potential reproductive individuals, and the nestmate recognition results from cuticular hydrocarbons cues. However, data about the origin of that substance in Ponerinae ants are scarce. This study reports the occurrence of epidermal glands in the abdomen of the ant *Dinoponera lucida*. In this ant, the epidermis of the abdominal sternites has tall cells with well-developed nucleus contrasting with flattened and collapsed epidermis in the tergites, suggesting a glandular function in the epidermis of the sternites. The possible role of the glandular epidermis in the synthesis of cuticular hydrocarbons for the nestmate recognition is discussed. *Microsc. Res. Tech. 72:28–31, 2009.*

INTRODUCTION

Many Ponerinae ants lost a morphologic distinct queen caste so that all females may be potential reproductive individuals (Peeters et al., 1999), and consequently this can generate conflicts that engender a dominance hierarchy within the colony. In *Dinoponera quadriceps*, for example, only the dominant female, i.e., the gamergate or alpha, can mate, and it exhibits an exceptional dominance behavior bending her gaster forward, biting the tip of the antenna of a subordinate worker and rubbing it against its abdominal tergites (Monnin et al., 2002; Peeters et al., 1999). In ants, cuticular hydrocarbons play an important role for the recognition of nest, reproductive or hierarchical status of the ant, and egg discrimination (Cuvillier-Hot et al., 2001; Denis et al., 2006; Endler et al., 2004; Hannonen et al., 2002). Thus, like in other ant species, it is likely that cuticular hydrocarbons play a role in olfactory information in the recognition of the dominant female and nestmates in *D. quadriceps* (Monnin and Peeters, 1999; Monnin and Ratnieks, 1999; Peeters et al., 1999); 9-hentriacontene is the main cuticular hydrocarbon in the abdomen of the dominant female, but it is almost absent in subordinate workers (Monnin et al., 1998).

Although the blend of cuticular hydrocarbons has been well documented in the abdomen of *D. quadriceps*, there is doubt about glands that release these substances in the body cuticle, although Monnin et al. (2002) have pointed out that queen recognition in *D. quadriceps* is performed for secretions from Dufour glands. *D. lucida* is another Ponerinae with similar behavior to that showed by *D. quadriceps* (Peixoto et al., 2008). *D. lucida* is the largest known ant (rough 3 cm in length), and because it is restricted to Atlantic rainforest in Bahia and Espírito Santo States, Brazil, it make this species a threatened one due to deforestation.

The aim of this work was to evaluate the occurrence and morphology of epidermal glands in the abdomen of the Ponerinae ant *D. lucida*.

MATERIALS AND METHODS

Ten specimens of *D. lucida* were field collected in Itamaraju, state of Bahia, Brazil (license IBAMA 118/2004-CGFAU/LIC), and transferred alive to aqueous Bouin fixative solution.

The ants were dissected, and all individuals were subordinate workers since they had undeveloped ovaries and empty spermatheca. After the abdominal tergites and sternites were isolated, they were dehydrated in a graded ethanol series and embedded in historesin JB4. Some 5-μm-thick sections were stained with hematoxylin and eosin and another set of sections submitted to the histochemical tests PAS counter stain with hematoxylin, and mercury bromophenol blue at pH 2.5 to detect neutral carbohydrates and total protein, respectively (Pearse, 1984).

Another set of tergites and sternites, after ethanol dehydration were transferred to hexamethyldisilazane and air dried. These samples were then coated with a 30-nm gold layer and analyzed in a scanning electron microscope (SEM; LEO VP1430).

*Correspondence to: José Eduardo Serrão, Departamento de Biologia Geral, Universidade Federal de Viçosa, 36570-000 Viçosa, MG, Brazil.
E-mail: jeserrao@ufv.br

Received 16 May 2008; accepted in revised form 8 August 2008
Contract grant sponsors: CNPq, FAPEMIG, FAPESB/CNPq.
DOI 10.1002/jemt.20641
Published online 24 September 2008 in Wiley InterScience (www.interscience.wiley.com).

RESULTS

The SEM analyses showed that the surface of all tergites and sternites of *D. lucida* has no occurrence of pores that can be exocrine gland openings (Figs. 1A and 1B), which is confirmed by histological analyses, where there were not finding unicellular glands containing canals which open in the abdominal surface.

The epidermis of all tergites is a thin layer of collapsed cells (Fig. 2A), whereas in all sternites, the epidermis is a well-developed layer with tall cells (Fig. 2B). The epidermal cells of the sternites vary from cubic (Fig. 2C) to columnar (Fig. 2D), and their products should be released through pore canals of the cuticle, since excretory canals are lacking. The well-developed nucleus of these cells is spherical, containing decondensed chromatin (Figs. 3A and 3B). The basal cell region is folded with width canals reaching the middle cell region characterized by longitudinal grooves in the cytoplasm (Fig. 3B), whereas the apical cell region is filled with unstained granules (Fig. 3A), which were also negative for PAS and bromophenol blue tests (Figs. 3C and 3D).

The apical surface of the tall epidermis has basophilic material in the subcuticular space (Fig. 3B).

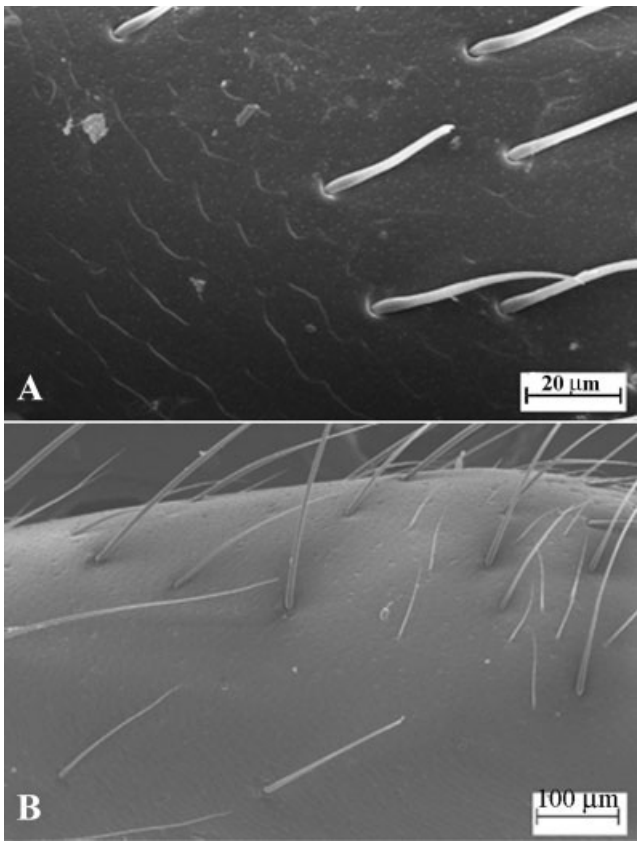

Fig. 1. Scanning electron micrographs of *Dinoponera lucida*. (**A**) Tergite surface. (**B**) Sternite surface.

DISCUSSION

An epidermis with tall cells like those found in the sternites of *D. lucida* workers is referred as playing a glandular role with their cells classified as type I, releasing its secretion across the cuticle (Noirot and Quennedey, 1991). The gland status of the epidermis in the sternites is supported by its thickness, basal infoldings and granules in the apical cytoplasm of the cell, and subcuticular space (Azevedo et al., 2007; Cruz-Landim and Abdalla, 2002), which was also observed in the antennal epidermis of *D. lucida* (Marques-Silva et al., 2006). A glandular epithelium in the sternites of *D. lucida* suggests that it may synthesize and/or transport the cuticular hydrocarbons used in the recognition of nestmates and/or of the dominant female such as reported in the queen of the ant *Megaponera foetens* (Hölldobler et al., 1994) and *D. quadriceps* (Monnin et al., 1998; Monnin and Ratnieks, 1999; Peeters et al., 1999). In solitary bees, the glandular epithelium is located in different abdominal segments, and because they do not use wax to nest building, the function of the glandular epithelium is uncertain, although it has been suggested that it plays a role in the cuticular hydrocarbon production (Guerino and Cruz-Landim, 2003).

The epidermis of *D. lucida* may transfer the hydrocarbons to the cuticle after production by the oenocytes that are the main site of hydrocarbons synthesis (Fan et al., 2003; Schal et al., 1998). Alternatively, the tall epithelium found in the sternites could play an important role in changing the cuticular hydrocarbons, and this further metabolism of cuticular hydrocarbons (or such resulting compounds) may be responsible for the production of different amounts or types of cuticular hydrocarbons between gamergate (dominant female) and workers, which in the later may be associated with nestmate recognition in *D. lucida*. In the ant *D. quadriceps*, the amount of 9-hentriacontene, main cuticular hydrocarbon in the dominant female, increases in the workers after removing the dominant ant from the nest (Peeters et al., 1999) However, workers have other cuticular hydrocarbons likely used in the nestmate recognition (Monnin et al., 1998; Peeters et al., 1999). It is suggested that hydrocarbons in insect are produced by oenocytes associated with either fat body or epithelial tissues (Dihel, 1975; Fan et al., 2003; Romer, 1980). Although Soroker and Hefetz (2000) stated that the fat body is the main site of synthesis of cuticular hydrocarbons in ants in these insects, the oenocytes are associated with fat body. Thus, the oenocytes may be classified as a class II gland (Noirot and Quennedey, 1991) that releases hydrocarbon into hemolymph associated with lipophorin that are transported to target tissues such as fat body, ovary, and cuticle, presumably through epidermal cells (Fan et al., 2002; Schal et al., 1998).

Because the dominant female of *D. quadriceps* rub the antennae of a subordinate worker in its tergites V and VI, the Dufour gland does not produce cuticular hydrocarbons (Monnin et al., 1998). On the other hand, Monnin et al. (2002) stated that contents from Dufour gland play a role in the gamergate recognition. In the workers analyzed here, the epidermis of all tergites has no features of a gland, but a future study of abdominal tergites in the dominant female will be useful to

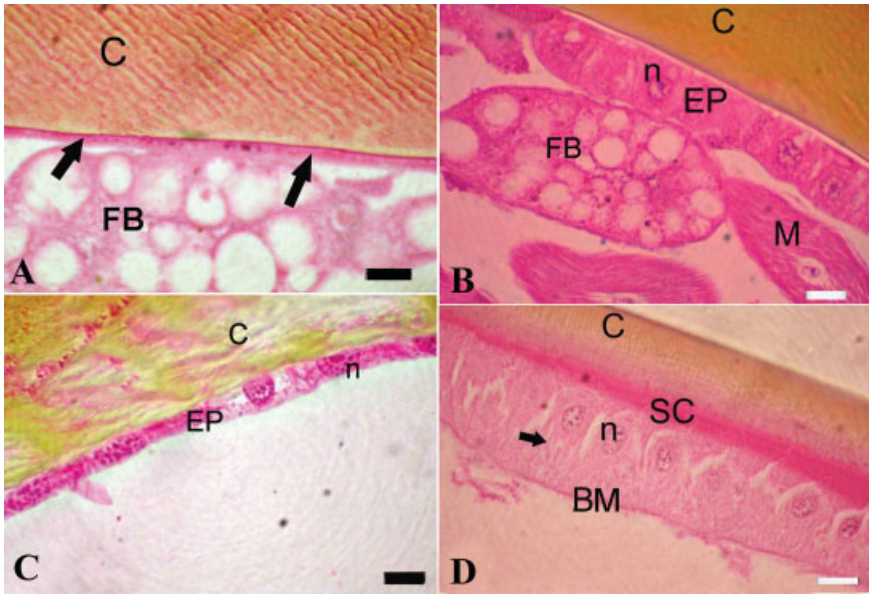

Fig. 2. Histological sections of the integument of *Dinoponera lucida*. (**A**) Tergite III showing flattened epidermis (arrows). (**B**) Sternite I showing epidermis with tall cells (EP) containing well-developed spherical nucleus (n). (**C**) Sternite II showing epidermis with cubic cells (EP) and nucleus with decondensed chromatin (n). (**D**) Sternite V showing columnar cells with well-developed nucleus (n) and infoldings in the basal cell region (arrow). SC, subcuticular space; BM, basal membrane; C, cuticle; FB, fat body; M, muscle. Scale bars = 10 μm.

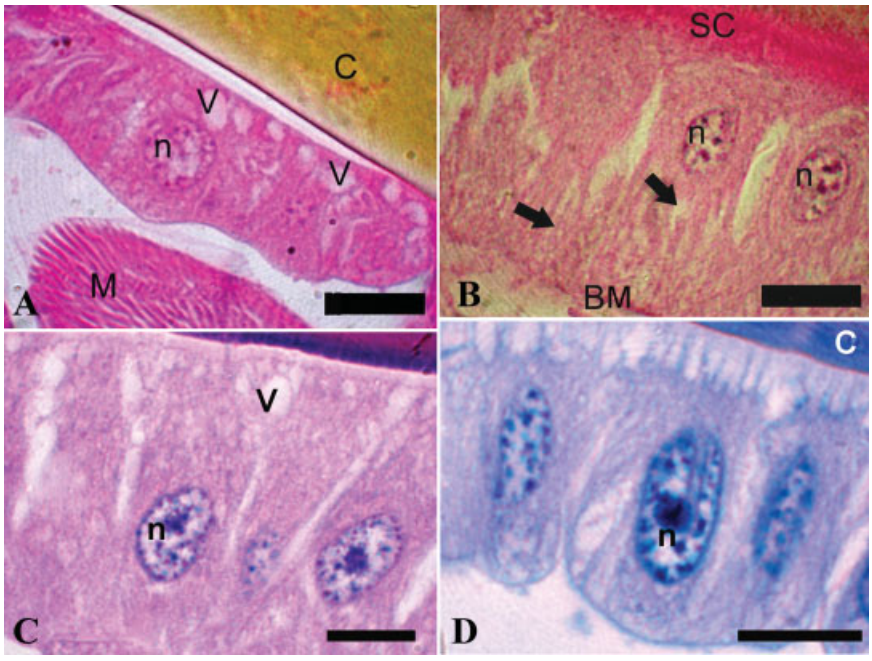

Fig. 3. Histological sections of the integument of *Dinoponera lucida*. (**A**) Sternite I showing epidermal cells with vacuoles (V). (**B**) Sternite III showing epidermal cells with infoldings (arrows) near to the basal membrane (BM), which almost reach the median–apical portion of the cell. SC, subcuticale space. (**C**) Epidermis of the sternite V reacted by PAS test. Note the absence of glycogen deposits. (**D**) Epidermis of the sternite III stained with mercury bromophenol blue. Note weakly staining of the cell cytoplasm and nucleus (n). C, cuticle; M, muscle. Scale bars = 10 μm.

assure whether epidermal glands may have a similar effect to that of Dufour gland in the gamergate, contributing for the comprehension of the dominant behavior in *D. lucida*.

In conclusion, the presence of a well-developed epidermis in the sternites of the abdomen in *D. lucida* indicates that these cells play an important role in the release of cuticular substances.

ACKNOWLEDGMENTS

The authors are grateful to Dr. Riviane R. Hora for critical reading of the manuscript and to the Universidade Federal de Viçosa (Nucleus of Microscopy and Microanalyses) for making the scanning electronic microscope available.

REFERENCES

Azevedo DO, Teixeira EW, Alves MLT, Moreti ACC, Blochtein B, Zanuncio JC, Serrão JE. 2007. Comparative analyses of the abdominal tergal glands in *Apis mellifera* (Hymenoptera: Apidae) queens. Anim Biol 57:329–338.

Cruz-Landim C, Abdalla FC. 2002. Glândulas exócrinas das abelhas. Ribeirão Preto: FUNPEC. 181 pp.

Cuvillier-Hot V, Cobb M, Malosse C, Peeters C. 2001. Sex, age and ovarian activity affect cuticular hydrocarbons in *Diacamma ceylonense*, a queenless ant. J Insect Physiol 47:485–493.

Denis D, Blatrix R, Fresneau D. 2006. How ant manages to display individual and colonial signals by using the same channel. J Chem Ecol 32:1647–1661.

Dihel PA. 1975. Synthesis and release of hydrocarbons by the oenocytes of the desert locust, *Schistocerca gregaria*. J Insect Physiol 21:1237–1246.

Endler A, Liebig J, Schmitt T, Parker JE, Jones GR, Schreier P, Hölldobler B. 2004. Surface hydrocarbons of queen eggs regulate worker reproduction in a social insect. Proc Natl Acad Sci USA 101:2945–2950.

Fan Y, Chase J, Sevala V, Schal C. 2002. Lipophorin-facilitated hydrocarbon uptake by oenocytes in the German cockroach, *Blatella germanica*. J Exp Biol 205:781–790.

Fan Y, Zurek L, Dykstra MJ, Schal C. 2003. Hydrocarbon synthesis by enzymatically dissociated oenocytes of the abdominal integument of the German cockroach, *Blatella germanica*. Naturwissenschaften 90:121–126.

Guerino AC, Cruz-Landim C. 2003. Ocorrência e morfologia de glândulas tegumentares no abdome de algumas abelhas (Hymenoptera: Apidae): Um estudo comparado. Neotrop Entomol 33:261–267.

Hannonen M, Sledge MF, Turillazzi S, Sundström L. 2002. Queen reproduction, chemical signaling and worker behaviour in polygyne colonies of the ant *Formica fusca.* Anim Behav 64:477–485.

Hölldobler B, Peeters C, Obermayer M. 1994. Exocrine glands and the attractiveness of the ergatoid queen in the ponerine ant *Megaponera foetens*. Ins Soc 41:63–72.

Marques-Silva S, Matiello-Guss CP, Delabie JHC, Mariano CSF, Serrão JE. 2006. Exocrine antennal glands of the ant *Dinoponera lucida* (Formicidae: Ponerinae). Microsc Res Tech 69:885–890.

Monnin T, Peeters C. 1999. Dominance hierarchy and reproductive conflicts among subordinates in a monogynous queenless ant. Behav Ecol 10:323–332.

Monnin T, Ratnieks FLW. 1999. Reproduction versus work in queenless ants: When to join a hierarchy of hopeful reproductives? Behav Ecol 46:413–422.

Monnin T, Malosse C, Peeters C. 1998. Solid-phase microextraction and cuticular hydrocarbon differences related to reproductive activity in queenless ant (*Dinoponera quadriceps*). J Chem Ecol 24:473–490.

Monnin T, Ratnieks FLW, Jones GR, Beard R. 2002. Pretender punishment induced by chemical signaling in a queenless ant. Nature 419:61–65.

Noirot C, Quennedey A. 1991. Glands, gland cells, glandular units: Some comments on terminology and classification. Ann Soc Ent France 27:123–128.

Pearse AGE 1984. Histochemistry: Theoretical and applied. London: J. & A. Churchil. 484 pp.

Peeters C, Monnin T, Malosse C. 1999. Cuticular hydrocarbons correlated with reproductive status in a queenless ant. Proc R Soc London B 266:1323–1327.

Peixoto AV, Campiolo S, Lemes TN, Delabie JHC, Hora RR. 2008. Behavior and reproductive structure of the ant *Dinoponera lucida* Emery (Hymenoptera: Formicidae). Rev Bras Ent 52:88–94.

Romer F. 1980. Histochemical and biochemical investigations concerning the function of larval oenocytes of *Tenebrio molitor* L. (Coleoptera: Insects). Histochimie 69:69–84.

Schal C, Sevala VL, Young HP, Bachmann JAS. 1998. Synthesis and transport of hydrocarbons: Cuticle and ovary as target tissues. Am Zool 38:382–393.

Soroker V, Hefetz A. 2000.Hydrocarbon site of synthesis and circulation in the desert ant *Cataglyphis niger.* J Insect Physiol 46:1097–1102.