

Chemical composition of the intramandibular glands of the ant *Neoponera villosa* (Fabricius, 1804) (Hymenoptera: Ponerinae)

Luiza Carla Barbosa Martins · Fabio Santos Do Nascimento ·
Maria Cláudia Guidetti Campos · Eraldo Rodrigues Lima ·
José Cola Zanuncio · José Eduardo Serrão

Received: 29 April 2014 / Accepted: 30 September 2014 / Published online: 11 October 2014
© Springer Basel 2014

Abstract Products of exocrine glands modulate the behavior of social insects. Among the exocrine glands, the function of intramandibular glands has not been well characterized in social Hymenoptera. To study the effects of exocrine gland secretions on the behavior of the ant, *Neoponera villosa*, identification of cuticular and intramandibular gland compounds was performed. Fifteen different compounds were identified in workers and queens of *N. villosa*. Linear alkanes C₂₆, C₂₇, C₂₈, C₃₀ and C₃₆, as well as the methyl alkanes 13-, 11- MeC₂₈, 3 MeC₂₉, 13-, 11- MeC₃₂, 13-, 11- MeC₃₃, 13- MeC₃₆, 13,17-; 15,19-DiMeC₃₇ and 18-, 17-, 13-MeC₃₈ and esters triacontyl acetate were found on the

body and mandible surface of both workers and queens. The sterols, cholesterol and sitosterol were found only in the mandibles, with cholesterol present in both workers and queens, and sitosterol present only in queens. The results suggest that intramandibular gland compounds of *N. villosa* may play a role in worker activity. The presence of hydrocarbons and cholesterol in workers and sitosterol in the mandible of queens may be associated with caste profile.

Keywords Formicidae · Exocrine gland · Chemical communication · Pheromone · Sterol · Acetate

Handling Editor: Stephen J. Martin.

L. C. B. Martins · J. E. Serrão (✉)
Departamento de Biologia Geral, Universidade Federal de Viçosa, Av. Peter Henry Rolfs s/n-Campus Universitário-MG, Viçosa, MG 36570-000, Brazil
e-mail: jeserrao@ufv.br

L. C. B. Martins
e-mail: luiza.martins@ufv.br

F. S. Do Nascimento · M. C. G. Campos
Departamento de Biologia da Faculdade de Filosofia Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, Av. Bandeirantes, Ribeirão Preto, São Paulo 3900, Brazil
e-mail: fsnascim@usp.br

M. C. G. Campos
e-mail: mclaudiac@bol.com.br

E. R. Lima · J. C. Zanuncio
Departamento de Entomologia, Universidade Federal de Viçosa, Viçosa, MG 36570-000, Brazil
e-mail: mothman@ufv.br

J. C. Zanuncio
e-mail: zanuncio@ufv.br

Introduction

The genus *Pachycondyla* was revised for the New World by Mackay and Mackay (2010) and currently this diverse genus was divided into 19 genera (Schmidt and Shattuck 2014). Thus, the former *Pachycondyla foetida* species complex is now *Neoponera foetida* with 13 species for the Neotropics, from which seven species occur in Brazil, including *Neoponera villosa* (Fernandes et al. 2014; Schmidt and Shattuck 2014). These ants are relatively large with length size, between 1.5 and 2 cm, and exhibit arboreal nesting habits (Lucas et al. 2002, Wild 2002). Some species of *Neoponera* are generalist scavengers and predators of arthropods (Orivel and Dejean 2001).

The social behavior of insects depends on reciprocal communication and cooperation, which is a basic requirement for maintaining the integrity of colonies (Wilson 1971; Hölldobler and Wilson 1990; Marques-Silva et al. 2006). Social communication among insects may be facilitated by pheromones that modulate behavioral responses (Jackson and Morgan 1993; Blomquist and Bagnères 2010).

The knowledge of morphological and physiological characteristics of the exocrine glands in social insects is important for the comprehension of behavioral and evolutionary aspects of these insects. A common characteristic of social insects is the diversity of exocrine glands distributed throughout the body (Billen 2008). In ants, there are 75 exocrine glands described (Billen 2009), which vary in their morphological, chemical and functional complexity (Billen and Morgan 1998; Caetano et al. 2002). Some glands of ants release pheromones, which are important for communication in social insects (Billen 2009).

While mandibular glands have been well studied, particularly with regard to release of chemical compounds, our knowledge of intramandibular glands is restricted to morphological characteristics in different insects (Santos et al. 2009; Martins and Serrão 2011; Billen and Delsinne 2013). To date, none of the chemical compounds secreted by these glands has been identified and this study is the first report on the chemical composition of intramandibular gland in ants.

The primary function of cuticular hydrocarbons is to prevent water loss (Singer et al. 1998; Monnin 2006). However, they may also play a role in chemical communication between social insects (Singer et al. 1998; Monnin 2006; Hora et al. 2010). These compounds act as both “primers”, changing insect physiology, (Le Conte and Hefetz 2008) and “releasers”, resulting in rapid and reversible changes in the behavior of the insect (Wilson 1963; Vilela and Della Lucia 2001). In this regard, it is well established that cuticular hydrocarbons are involved in sex, aggregation, dispersion, alarm, territoriality, trail marking, oviposition, and recognition of nestmates and castes.

Behavioral responses stemming from chemical communication occur as a result of the large diversity of specialized exocrine glands, which vary in their structure, chemical composition, and physiology (Billen and Morgan 1998; Caetano et al. 2002).

In the ant *N. villosa*, intramandibular glands have two cell types, viz., gland class I characterized by cuboidal epidermal cells and class III characterized by isolated cells in the internal cavity of the mandible with presence of canaliculi that open in pores on the mandible surface (Martins and Serrão 2011). To delineate the possible functions of the intramandibular glands in ants, we identified the cuticular and intramandibular gland compounds.

Materials and methods

Ants

Five colonies of *N. villosa* were collected in the experimental field of the Cocoa Research Center (CEPLAC), Ilhéus, state of Bahia, Brazil at geographic coordinates

14°45' S and 39°13' W. The ants were reared in the laboratory within artificial nests at least 3 months before analysis. Nests were maintained at 25 ± 4 °C and about 50–80 % relative humidity.

The ants were identified by Dr. Jacques H. C. Delabie and voucher specimens were deposited in the Laboratory of Myrmecology, Centre for Cocoa Research (CEPEC), Ilhéus, Bahia, Brazil.

All colonies were fed ad libitum on an identical diet of honey and the beetle *Tenebrio molitor* larvae. Queens and workers were collected directly from the colonies.

Extraction of cuticular and intramandibular gland compounds

Artificial nests were transported and maintained in the laboratory at same conditions of the reared laboratory, and the ants were taken directly from the nest. For the extraction and characterization of the body and mandibular cuticular chemical profile in *N. villosa*, four physogastric queens and three workers were obtained from each of three colonies. Ants were removed from colonies using clean tweezers previously washed with hexane.

All samples of the cuticular and mandibular chemical profiles of queens and workers were obtained by a non-destructive technique using solid phase microextraction (SPME).

The extraction of chemical compounds was performed by a slight, four second, friction contact of a polydimethylsiloxane fiber (30 µm) (Supelco) with the dorsal surface of the head, thorax, and abdomen of the ant. Afterwards, mandibles were removed from the body and macerated in glass vials with the aid of a glass rod; all of them washed twice in Extran detergent, Milli-Q water and hexane. Subsequently, slight friction of the polydimethylsiloxane fiber with the maceration was performed for 10 s. The fibers were immediately used for gas chromatography and mass spectrometry analyzes.

After the extraction of chemical compounds, the queens were dissected and only those with activate ovaries and with spermatozoa into the spermathecae were used in the chemical compound analyzes.

Gas chromatography and mass spectrometry (GC–MS)

Analysis of samples from polydimethylsiloxane fibers was performed at the Laboratory of Behavior and Ecology of Social Insects, Universidade de São Paulo, Ribeirão Preto, São Paulo, Brazil. The analyzes were conducted with a SHIMADZU GC–MS QP2010. Separation was achieved on Rtx-5MS column (30 m), 0.25 mm of the internal diameter and 0.25 µm of the film thickness; using helium as the carrier gas at 1.0 mL/min. The SPME fibers were

mounted directly in the GC–MS for 4 min to desorb the compounds. The temperature in the injector port was 250 °C. The initial temperature in the oven (initial column temperature) was 150 °C, increasing 3 °C/min until 280 °C (held for 10 min), after, increasing by 10 °C/min until it reaches 300 °C (held for 5 min). Analyzes were performed in splitless mode. The mass spectra were obtained by 70 eV ionization.

Data were analyzed by mass spectral characterization. Measurements were based on peak areas obtained from the chromatograms. Data were analyzed with the computer program, GC–MS Solutions for Windows (Shimadzu Corporation) and the chemical compounds were identified based on their mass spectra using diagnostic ions as well compared to the mass spectral library Nist 08 database.

Results

The GC–MS analyzes of the mandible and cuticle revealed the presence of 15 different compounds in both workers and queens of *N. villosa* (Table 1). Linear alkanes C₂₆, C₂₇, C₂₈, C₃₀ and C₃₆, as well as the methyl alkanes 13-, 11-MeC₂₇, 3-MeC₂₉, 13-, 11-MeC₃₂, 13-, 11-MeC₃₃, 13-MeC₃₆, 13,17-, 15,19-DiMeC₃₇ and 18-, 17-, 13-MeC₃₈ and ester triacontyl acetate (Fig. 1; Table 1), the precursors

of sterol hormones, cholesterol and sitosterol were found only in the mandible of *N. villosa*, with cholesterol in workers and queens and sitosterol only in queens were identified (Fig. 1; Table 1).

Discussion

The linear and branched alkanes found on the cuticle and mandible of *N. villosa* are long-chain compounds between 26 and 38 carbons, similar to those found in other social insects such as termites (Weil et al. 2009), bees (Abdalla et al. 2003, 2004; Dani et al. 2005), wasps (Panek et al. 2001), and other ants (D’Ettorre et al. 2004; Martin and Drijfhout 2009; Wilgenburg et al. 2011). These long-chain cuticular hydrocarbons have been considered important for nestmate recognition in *Iridomyrmex purpureus* (Thomas et al. 1999), *Cataglyphis niger* (Lahav et al. 1999) and *Pogonomyrmex barbatus* (Wagner et al. 2000). Long-chain hydrocarbons were also found on the cuticle and in the post-pharyngeal gland of the ant *N. villosa*, suggesting an important role in nestmate recognition (Lucas et al. 2004).

The 15 compounds found in the mandible and body cuticle of *N. villosa* in this study were lower than 82 once found in this same ant species by Lucas et al. (2002), (2004). Different from our study, these authors extracted

Table 1 Chemical compounds identified by GC/MS of intramandibular glands (M) and of body surface (B) of workers and queens of *Neoponera villosa*

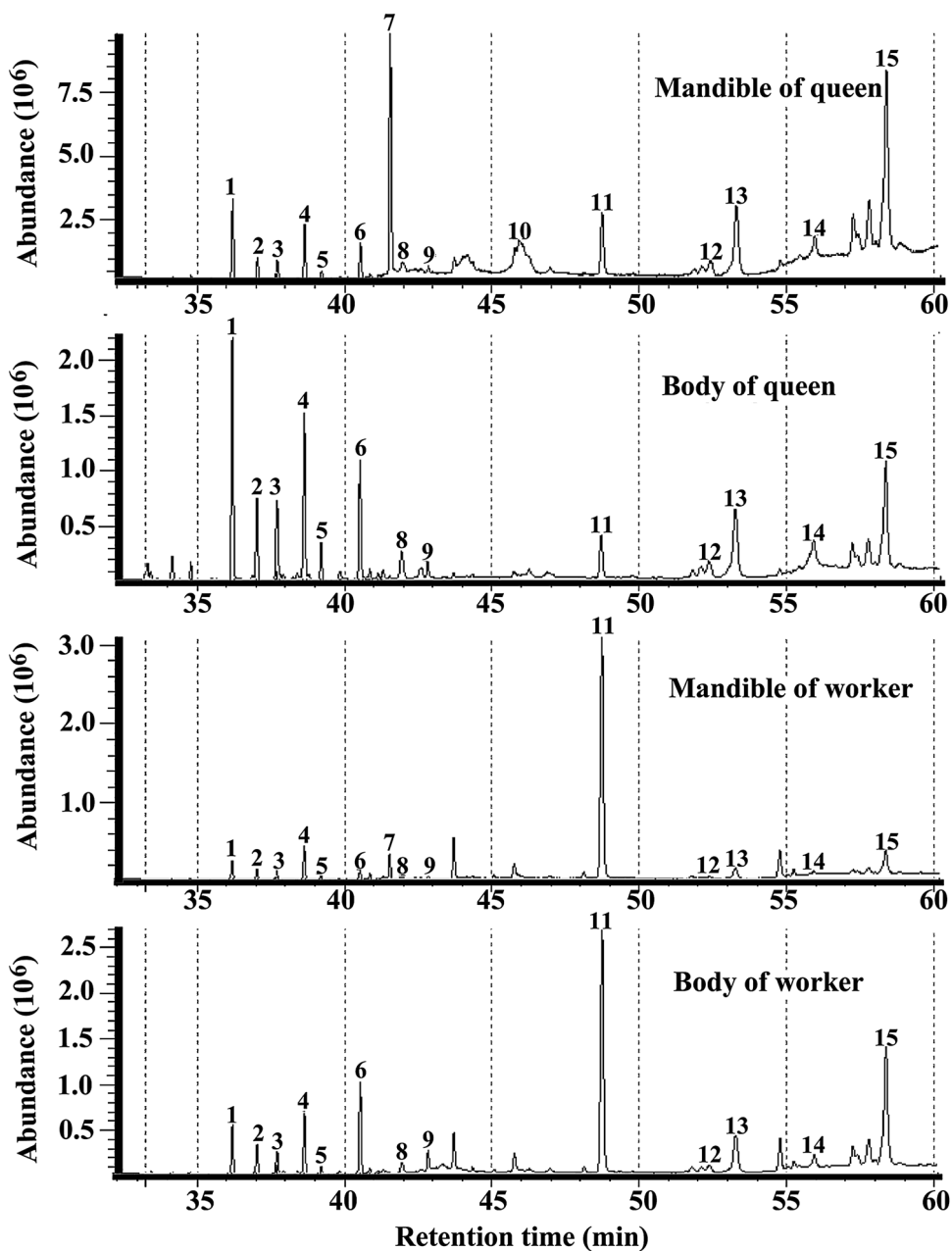
Peak	RT	Compound	Queen		Worker	
			M (n = 4)	B (n = 4)	M (n = 6)	B (n = 6)
1	36.213	C ₂₆	4.88 ± 3.43	9.32 ± 6.96	6.14 ± 5.00	12.86 ± 6.58
2	37.038	C ₂₇	1.49 ± 0.76	3.18 ± 2.19	2.37 ± 1.79	5.60 ± 2.74
3	37.718	13-, 11-MeC ₂₇	2.13 ± 1.13	6.39 ± 1.10	1.67 ± 1.50	3.75 ± 2.17
4	38.646	C ₂₈	2.33 ± 2.49	6.80 ± 4.37	7.49 ± 5.21	14.71 ± 7.49
5	39.214	3-MeC ₂₉	0.51 ± 0.51	1.23 ± 1.15	0.68 ± 0.64	2.12 ± 1.07
6	40.533	C ₃₀	3.51 ± 1.15	4.18 ± 3.73	3.20 ± 3.41	6.83 ± 3.19
7	41.546	Cholesterol	10.04 ± 9.16	–	13.03 ± 14.46	–
8	41.934	13-, 11-MeC ₃₂	0.97 ± 0.81	2.13 ± 1.65	0.76 ± 0.51	1.79 ± 0.77
9	42.834	13-, 11-MeC ₃₃	0.44 ± 0.17	0.56 ± 0.50	0.55 ± 0.46	1.42 ± 0.41
10	45.905	Sitosterol	1.52 ± 1.64	–	–	–
11	48.709	Triacontyl acetate	6.57 ± 3.52	2.27 ± 1.75	55.31 ± 3.17	38.77 ± 10.06
12	52.355	C ₃₆	2.02 ± 1.11	2.41 ± 0.40	0.20 ± 0.25	1.05 ± 0.69
13	53.247	13-MeC ₃₆	10.82 ± 1.05	6.99 ± 6.14	2.40 ± 2.69	6.91 ± 2.55
14	55.923	13,17-, 15,19-DiMeC ₃₇	1.92 ± 0.61	1.64 ± 1.54	0.42 ± 0.44	1.01 ± 0.43
15	58.355	18-, 17-, 13-MeC ₃₈	17.76 ± 15.61	9.66 ± 8.18	13.88 ± 8.65	15.05 ± 6.11

Relative proportions (mean ± SD)

Identification of compound and their respective diagnostic ions: Peaks, 1: 366; 2: 380; 3: 196/197, 224/225, 168/169, 252/253; 379; 4: 394; 5: 364/365; 392/393; 6: 422; 7: 386; 8: 196/197, 295/296; 168/169; 449; 9: 224/225, 280/281, 196/197; 463; 10: 414; 11: 420; 12: 506; 13: 196/197; 505; 14: 224/225, 280/281, 295/296, 351/352, 196/197, 267/268, 308/309; 519; 15: 266/267, 308/309; 252/253, 322/323, 378/379, 196/197; 533
RT retention time (min)

Fig. 1 Chromatogram of the chemicals found in the mandible and body surface of *Neoponera villosa* queens and workers.

Peaks 1 C₂₆, 2 C₂₇, 3 13-, 11-MeC₂₇, 4 C₂₈, 5 3-MeC₂₉, 6 C₃₀, 7 Cholesterol, 8 13-, 11-MeC₃₂, 9 13-, 11-MeC₃₃, 10 Sitosterol, 11 Triacontyl acetate, 12 C₃₆, 13 13-MeC₃₆, 14 13,17-, 15,19-DiMeC₃₇, 15 18-, 17-, 13-MeC₃₈



cuticular hydrocarbons from whole body by the immersion of the ant for 5 min in 200 μ L of hexane with three-dimensional agitation, which may be resulted in the extraction of compounds of internal organs including lipids of cell membrane which are rich in linear and branched hydrocarbons Alberts et al. 2010).

An interesting finding was the presence of cholesterol and sitosterol in the mandible of *N. villosa*. Among the predatory wasps and ants, sterols are found in large quantities in the tissues, which are linked with the dietary sterols from their prey (Behmer and Nes 2003). Sterols have three critical functions in insects: (1) they are important components of cell membranes; (2) they are precursors for the

molting hormone, ecdysone (Koolman 1990; Grieneisen 1994), and (3) they play a role in regulating genes involved in insect developmental (Porter et al. 1996). However, insects are not able to synthesize sterols and have to ingest exogenous sterols from food resources (Clark and Bloch 1959; Svoboda et al. 1994).

In social bees, the main sterols from pollen grains were also found in the mandibular, hypopharyngeal, and head salivary glands (Ferreira-Caliman et al. 2012). Svoboda et al. (1986) showed that sterols are present in hypopharyngeal and in mandibular glands of honeybee workers. Steroids were found on the cuticle of the parabiotic ants, *Crematogaster modiglianii* and *Camponotus ruffifemur*, as

well as in their dufour glands, suggesting that these compounds are produced in the dufour gland and then distributed to the body surface, providing an individual signature (Menzel et al. 2008). Ba et al. (1995) identified 26 steroids, including cholesterol and sitosterol, in eggs, larvae, workers, and queens of the ant *Solenopsis invicta*. In contrast, we found that intramandibular gland cholesterol and sitosterol were not transferred to the body surface of *N. villosa*, suggesting that both compounds found in the mandible are not directly involved in the production of cuticular signatures.

In *N. villosa*, hydrocarbons and cholesterol were present in workers and queens, while sitosterol was present in the mandible of queens alone. However, sitosterol may be involved in recognition of the physogastric queen, where it exclusively occurs in *N. villosa*, this occurrence may be associated with caste profile and labor division. Composition of the pheromones or the chemicals compounds varies between castes in ants (Do Nascimento et al. 1993; Hernández et al. 1999; Hughes et al. 2001; Cruz-Landim et al. 2011). Intra-nest variability of cuticular hydrocarbon profiles in ants has been shown to be linked with differences in the tasks performed by workers (Bonavita-Cougourdan et al. 1987, 1993), and with reproductive status (Peeters et al. 1999; Liebig et al. 2000; Cuvillier-Hot et al. 2001; D'Ettore et al. 2004; Lommelen et al. 2006). Cuticular hydrocarbons are similar between castes of *N. villosa*, although they have different concentrations. In this way, sitosterol occurs only in the mandibles of queen, suggesting that the intramandibular glands of queens and workers of this ant have different functions, although the significance of this remains unknown.

Another interesting result is the presence of long-chain acetate found on the body and mandible surfaces of both workers and queens of *N. villosa*. Long-chain acetate has different functions in different insects: sex and aggregation pheromones in Lepidoptera and *Drosophila*, respectively (Tschuch et al. 2005). Defensive functions can be found in bumble bees *Bombus* and *Psithyrus rupestris* (Wheeler and Duffield 1988; Zimma et al. 2003), tenthredinid sawfly (Jonsson et al. 1988) and in the Thysanoptera *Suoce-rathrips linguis* (Tschuch et al. 2005). Acetates and other compounds are found together with hydrocarbons in the dufour glands of some ants (Attygalle and Morgan 1984; Jackson et al. 1989) functions as repellents against predators (Tschuch et al. 2005).

The alkane, acetate and steroid profile of *N. villosa* in queen and workers suggests that some of these compounds in the intramandibular glands may be linked with caste profile, labor division and caste recognition. Although long-chain hydrocarbons are relatively non-volatile (Blomquist and Bagnères 2010), the action of these compounds as pheromones may occur via contact between

individuals of the colonies, as ants often allow physical contact during hygiene behavior (allo-grooming) and trophallaxis (Hefetz 2007). In addition to nestmate recognition, cuticular hydrocarbons also play a role in regulating reproduction in ants (Peeters et al. 1999; Liebig et al. 2000). However, because we did not find cuticular hydrocarbons exclusively in *N. villosa* queens, this reproductive function seems not to be carried out by hydrocarbons from intramandibular gland, although sitosterol present only in queens may play a similar role.

Our study show that sitosterol and cholesterol were present in intramandibular glands, but they are lacking in the body cuticle. Moreover, we found large amounts of cholesterol and sitosterol in intramandibular gland in queens. However, despite these findings indicating an important role for sterols in different castes, their functions remain unknown.

Acknowledgments This research was supported by Brazilian research agencies Coordination of Improvement of Higher Education Personnel (CAPES), Minas Gerais State Research Agency (FAP-EMIG), National Council of Research (CNPq), National Program for Excellence (PRONEX SECTI-FAPESB/CNPq—PNX 0011/2009), São Paulo State Research Agency (FAPESP 2010/10027-5) and National Institute of Science and Technology of Semiochemicals in Agriculture (INCT). Authors are grateful to Mr. José Raimundo Maia and Mr. José Adade for their assistance in the ant collection (Laboratory of Myrmecology the Cocoa Research Center, Ilhéus, Bahia, Brazil).

References

- Abdalla FC, Jones GR, Morgan ED, Cruz-Landim C (2003) Comparative study of the cuticular hydrocarbon composition of *Melipona bicolor* Lepeletier, 1836 (Hymenoptera, Meliponini) workers and queens. *Genet Mol Res* 2:191–199
- Abdalla FC, Jones GR, Morgan ED, Cruz-Landim C (2004) Chemical composition of the Dufour gland secretion in queens of *Melipona bicolor* (Hymenoptera, Meliponini). *J Braz Chem Soc* 15:621–625
- Alberts B, Bray D, Lewis J, Raff M, Roberts K, Walter P (2010) *Molecular biology of the cell*. Garland Science, New York
- Attygalle AB, Morgan ED (1984) Chemicals from the glands of ants. *Chem Soc Rev* 13:245–278
- Ba AS, Guo DA, Norton RA, Philips SA, Nes WD (1995) Developmental differences in the sterol composition of *Solenopsis invicta*. *Arch Insect Biochem Physiol* 29:1–9
- Behmer ST, Nes WD (2003) Insect sterol nutrition and physiology: a global overview. *Adv Ins Physiol* 31:1–72
- Billen J (2008) A novel exocrine gland in the trochanter of ant legs. *Acta Zool* 89:201–204
- Billen J (2009) Occurrence and structural organization of the exocrine glands in the legs of ants. *Arthropod Struct Dev* 38:2–15
- Billen J, Delsinne T (2013) A novel intramandibular gland in the ant *Tatuidris tatusia* (Hymenoptera: formicidae). *Myrmecol News* 19:61–66
- Billen J, Morgan ED (1998) Pheromone communication in social insects—sources and secretions. In: Vander Meer RK, Breed MD, Espelie KE, Winston ML (eds) *Pheromone communication in social insects: ants, wasps, bees, and termites*, Westview, Boulder, pp 3–33

- Blomquist GJ, Bagnères AG (2010) *Insect Hydrocarbons: Biology, Biochemistry, and Chemical Ecology*. Cambridge University Press, Cambridge
- Bonavita-Cougourdan A, Clement JL, Lange C (1987) Nestmate recognition: the role of cuticular hydrocarbons in the ant *Camponotus vagus* Scop. *J Entomol Sci* 22:1–10
- Bonavita-Cougourdan A, Clement JL, Lange C (1993) Functional subcaste discrimination (foragers and brood-tenders) in the ant *Camponotus vagus* Scop: polymorphism of cuticular hydrocarbon patterns. *J Chem Ecol* 9:1461–1477
- Caetano FH, Jaffé K, Zara FJ (2002) *Formigas: biologia e anatomia*. Rio Claro, Editora UNESP
- Clark AJ, Bloch K (1959) The absence of sterol synthesis in insects. *J Biol Chem* 234:2578–2582
- Cruz-Landim C, Gracioli-Vitti LF, Abdalla FC (2011) Ultrastructure of the intramandibular gland of workers and queens of the stingless bee, *Melipona quadrifasciata* (Meliponini). *J Ins Sci* 11:1–9
- Cuvillier-Hot VV, Cobb M, Malosse C, Peeters C (2001) Sex, age and ovarian activity affect cuticular hydrocarbons in *Diacamma ceylonense*, a queenless ant. *J Ins Physiol* 47:485–493
- D’Ettore P, Heinze J, Schulz C, Francke W, Ayasse M (2004) Does she smell like a queen? Chemoreception of a cuticular hydrocarbons signal in the ant *Pachycondyla inversa*. *J Exp Biol* 207:1085–1091
- Dani FR, Jones GR, Corsi S, Beard R, Pradella D, Turillazzi S (2005) Nestmate recognition cues in the honey bee: differential importance of cuticular alkanes and alkenes. *Chem Senses* 30:477–489
- Do Nascimento R, Morgan ED, Billen J, Schoeters E, Della-Lucia TMC, Bento JM (1993) Variation with the caste of the mandibular gland secretion in the leaf-cutting ant *Atta sexdens rubropilosa*. *J Chem Ecol* 19:907–918
- Fernandes IO, Oliveira ML, Delabie JHC (2014) Description of two new species in the Neotropical *Pachycondyla foetida* complex (Hymenoptera: Formicidae: Ponerinae) and taxonomic notes on the genus. *Myrmecol News* 19:133–163
- Ferreira-Caliman MJ, Silva CI, Mateus S, Zucchi R, Nascimento FS (2012) Neutral Sterols of Cephalic Glands of Stingless Bees and Their Correlation with Sterols from Pollen. *Psyche*. doi:10.1155/2012/982802
- Grieneisen ML (1994) Recent advances in our knowledge of ecdysteroid biosynthesis in insects and crustaceans. *Insect Biochem Mol Biol* 24:115–132
- Hefetz A (2007) The evolution of hydrocarbon pheromone parsimony in ants (Hymenoptera: Formicidae)—interplay of colony odor uniformity and odor idiosyncrasy. *Myrmecol News* 10:59–68
- Hernández JV, Cabrera A, Jaffé K (1999) Mandibular gland secretion in different caste of the leaf-cutter ant *Atta laevigata*. *J Chem Ecol* 25:2433–2444
- Hölldobler B, Wilson EO (1990) *The ants*. Belknap/Harvard University Press, Cambridge
- Hora RR, Delabie JHC, Santos CG, Serrão JE (2010) Glandular epithelium as a possible source of a fertility signal in *Ectatomma tuberculatum* (Hymenoptera: Formicidae) queens. *PLoS ONE* 5(4):e10219
- Hughes WOH, Howse PE, Goulson D (2001) Mandibular gland chemistry of grass-cutting ants: species, caste, and colony variation. *J Chem Ecol* 27:109–124
- Jackson BD, Morgan ED (1993) Insect chemical communication: pheromones and exocrine glands of ants. *Chemoeology* 4:125–144
- Jackson BD, Billen JJP, Morgan ED (1989) Dufour gland contents of three species of *Myrmecia* (Hymenoptera, Formicidae), primitive ants of Australia. *J Chem Ecol* 15:2191–2205
- Jonsson S, Bergstrom G, Lanne BS, Stensdotter U (1988) Defensive odor emission from larvae of two sawfly species, *Pristiphora erichsonii* and *P. wasmaeli*. *J Chem Ecol* 14:713–721
- Koolman J (1990) Ecdysteroids. *Zool Sci* 7:563–580
- Lahav S, Soroker V, Hefetz A, Vander Meer RK (1999) Direct behavioural evidence for hydrocarbons as ant recognition discriminators. *Naturwissenschaften* 86:246–249
- Le Conte Y, Hefetz A (2008) Primer pheromones in social hymenoptera. *Annu Rev Ent* 53:523–542
- Liebig J, Peeters C, Oldham NJ, Markstadter C, Hölldobler B (2000) Are variations in the cuticular hydrocarbons of queens and workers a reliable signal of fertility in the ant *Harpegnathos saltator*?. *Proc Natl Acad Sci USA* 97:4124–4131
- Lommelen E, Johnson CA, Drijfhout FP, Billen J, Wenseleers T, Gobin B (2006) Cuticular hydrocarbons provide reliable cues of fertility in the ant *Gnamptogenys striatula*. *J Chem Ecol* 32:2023–2034
- Lucas C, Fresneau D, Kolmer K, Heinze J, Delabie JHC, Pho DB (2002) A multidisciplinary approach to discriminating different taxa in the species complex *Pachycondyla villosa* (Formicidae). *Biol J Linn Soc* 75:249–259
- Lucas C, Pho DB, Fresneau D, Jallon JM (2004) Hydrocarbon circulation and colonial signature in *Pachycondyla villosa*. *J Ins Physiol* 50:595–607
- Mackay WP, Mackay E (2010) *The systematics and biology of the new world ants of the genus Pachycondyla* (Hymenoptera: Formicidae). Edwin Mellen Press, New York
- Marques-Silva S, Guss CPM, Delabie JHC, Mariano CSF, Zanuncio JC, Serrão JE (2006) Sensilla and secretory glands in the antennae of a primitive ant: *dinoponera lucida* (Formicidae, Ponerinae). *Microsc Res Tech* 69:885–890
- Martin S, Drijfhout F (2009) A Review of Ant Cuticular Hydrocarbons. *J Chem Ecol* 35:1151–1161
- Martins LCB, Serrão JE (2011) Morphology and histochemistry of the intramandibular glands in Attini and Ponerini (Hymenoptera, Formicidae) species. *Microsc Res Tech* 74:763–771
- Menzel F, Blüthgen N, Schmitt T (2008) Tropical parabiotic ants: highly unusual cuticular substances and low interspecific discrimination. *Front Zool* 5:1–16
- Monnin T (2006) Chemical recognition of reproductive status in social insects. *Ann Zool Fenn* 43:515–530
- Orivel J, Dejean A (2001) Comparative effect of the venoms of ants of the genus *Pachycondyla* (Hymenoptera: ponerinae). *Toxicon* 39:195–201
- Panek LM, Gamboa GJ, Espelie KE (2001) The effect of a wasp’s age on its cuticular hydrocarbon profile and its tolerance by nestmate and non-nestmate conspecifics (*Polistes fuscatus*, Hymenoptera: vespidae). *Ethology* 107:55–63
- Peeters C, Monnin T, Malosse C (1999) Cuticular hydrocarbons correlated with reproductive status in a queenless ant. *Proc R Soc Lond B Biol Sci* 266:1323–1327
- Porter JA, Young KE, Beachy PA (1996) Cholesterol modification of Hedgehog signaling proteins in animal development. *Science* 274:255–259
- Santos CG, Megiolaro F, Serrão JE, Blochtoin B (2009) Morphology of the head salivary and intramandibular glands of the stingless bee *Plebeia emerina* (Friese) (Hymenoptera, Meliponini) workers associated with propolis. *Ann Ent Soc Am* 102:137–143
- Schmidt CA, Shattuck SO (2014) The higher classification of the ant subfamily ponerinae (Hymenoptera: formicidae), with a review of ponerine ecology and behavior. *Zootaxa* 3817:001–242
- Singer TL, Espelie KE, Gamboa GJ (1998) Nest and nestmate discrimination in independent-founding wasps. In: Vander Meer RK, Breed MD, Winston ML, Espelie EK (eds) *Pheromone communication in social insects*. Westview, Boulder, pp 57–78

- Svoboda JA, Herbert EW, Thompson MJ, Feldlaufer MF (1986) Selective sterol transfer in the honey bee: its significance and relationship to other hymenoptera. *Lipids* 21:97–101
- Svoboda JA, Feldlaufer MF, Weirich GF (1994) Evolutionary aspects of steroid utilization in insects. *ACS Symp Ser* 562:126–139
- Thomas ML, Parry LJ, Allan RA, Elgar MA (1999) Geographic affinity, cuticular hydrocarbons and colony recognition in the Australian meat ant *Iridomyrmex purpureus*. *Naturwissenschaften* 86:87–92
- Tschuch G, Lindemann P, Niesen A, Csuk R, Moritz G (2005) A novel long-chained acetate in the defensive secretion of thrips. *J Chem Ecol* 31:1555–1565
- Vilela EF, Della Lucia TNC (2001) Feromônios de insetos: biologia, química e emprego no manejo de pragas. Ribeirão Preto, Holos
- Wagner D, Tissot M, Cuevas W, Gordon DM (2000) Harvester ants utilize cuticular hydrocarbons in nestmate recognition. *J Chem Ecol* 26:2245–2257
- Weil T, Hoffmann K, Kroiss J, Strohm E, Korb J (2009) Scent of a queen-cuticular hydrocarbons specific for female reproductives in lower termites. *Naturwissenschaften* 96:315–319
- Wheeler JW, Duffield RM (1988) Pheromones of Hymenoptera and Isoptera. In: Morgan ED, Mandava NB (eds) *Handbook of Natural Pesticides*, 4th edn. CRC Press, Boca Raton, FL, Pheromones, pp 59–206
- Wild AL (2002) The genus *Pachycondyla* (Hymenoptera: formicidae) in Paraguay. *Bol Mus Nac Hist Nat Parag* 14:1–18
- Wilgenburg EV, Symonds MRE, Elgar MA (2011) Evolution of cuticular hydrocarbon diversity in ants. *J Evol Biol* 24:1188–1198
- Wilson EO (1963) The social biology of ants. *Annu Rev Ent* 8:345–368
- Wilson EO (1971) The insect societies. Harvard Univ. Press, Cambridge
- Zimma BO, Ayasse M, Tengo J, Ibarra F, Schulz C, Francke W (2003) Do social parasitic bumblebees use chemical weapons? (Hymenoptera, Apidae). *J Comp Physiol A* 189:769–775