Metapleural- and Postpharyngeal-Gland Secretions from Workers of the Ants *Solenopsis invicta* and *S. geminata*

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Chemical analyses by GC–MS of the metapleural glands (MG) from workers of *Solenopsis invicta* and *S. geminata* revealed for the first time the chemical composition of these glands and showed small differences between the two species. The MG of both species contain oleic, stearic, linoleic, and palmitic acid. Both ants, in addition, have small but significant amounts of hydrocarbons in their MG reservoir, which are the same as those found in their postpharyngeal glands (PPG). The PPG of both species contain alkanes, alkenes, and Me-branched alkanes. Each species is characterized by a specific composition of PPG chemicals with some overlap between species. These results suggest that the MG synthesizes mainly palmitic, linoleic, oleic, and stearic acids in these two ants, whereas PPG contains hydrocarbon mixes that widely vary between these two phylogenetically related species.

Introduction. – Most ants are known to have highly developed metapleural[1]) glands (MG) located laterally on each side of the metathorax of workers and queens. This pair of symmetrical glands consists of secretory cells that secrete their product into a reservoir, which has an opening to the outside [1][2]. The anatomy of the gland is such that any secretion collected by the reservoir is continuously spilled to the outside, bathing the ant body and its surroundings with the secretion. The function of this secretion has been postulated as related to nestmate-recognition signals [3]. *Jaffe* and *Puche* [4] showed that, in the case of *Solenopsis geminata*, the MG secretions were used by workers as a colony-specific territorial marker. Other authors suggested that, in most ants, these secretions consist of antiseptic chemicals (see, *e.g.*, [5]), although, in some ants, it may serve as an alarm pheromone [3]. Chemically, the secretion has been analyzed in some ant species, and the compounds found were compatible with the antiseptic hypothesis [6–13] and with that of alarm pheromones in *Crematogaster inflata* [14]. All these findings indicate that MG secretion may have a variety of functions. These may differ between species, but, in most cases, these glands are likely to secrete antiseptic chemicals.

Postpharyngeal glands (PPG) are a pair of branched, tubular structures that occupy a large portion of the head overlaying the brain. Much controversy exists about the possible functions of these glands, as they participate in the digestion of fats, the absorption of fatty acids and glycerol esters, the regulation of caste determination [15],

[1]) Or *metasternal* glands.

and as the *Gestalt* organ for nestmate recognition [16]. *Bagnères* and *Morgan* [17] suggested that PPG may serve a '*purely mechanical purpose in providing lubrication and a softener for the cuticular wax*', in addition to a possible social function.

To gain more insight into the adaptive significance of MP and PPG in ants, we here report the chemical composition of MP secretions from the workers of the Myrmicinae, *Solenopsis invicta* and *S. geminata*. Due to the chemical nature of the compounds found, we also analyzed the chemical composition of their PPG and compared it with that of the MG. Both ant species are ecologically dominant wherever they occur, and are considered major pest species in some places.

Experimental. – Workers of the Myrmicinae, *Solenopsis invicta* and *S. geminata*, were collected in Florida, USA, and in Valle de Sartenejas, Estado Miranda, Venezuela, respectively. Both were brought to the laboratory, where they were maintained as laboratory colonies in Florida and Venezuela, respectively. Bodies of workers were frozen for a few minutes and immobilized, and the MG reservoir was extracted by dissecting the glands from ten workers of *S. invicta* and from 20 workers of *S. geminata*, by cutting the metathorax, extracting the gut and fatty bodies, and then dissecting the part of the cuticle where the gland is located. Dissection of the PPG from 20 individuals of both species was achieved by removing the mandibles with a forceps, and pulling the glands through the resulting opening with a forceps. Additionally, worker bodies from both species were immersed in 0.5 ml of CH_2Cl_2 during 10 min to obtain body washes for further chemical analysis. Extract of worker's whole bodies were obtained by placing ant bodies in CH_2Cl_2 for over 24 h. Venom glands from ten individuals of *S. invicta* were dissected and extracted with CH_2Cl_2. Glass capillaries containing MG secretions, or metapleural, or postpharyngeal, or venom glands, were placed in 0.5 ml of CH_2Cl_2. An internal standard, consisting of 50 µl of heptadecane soln. (70 ng/µl), was added to the gland extracts. These extracts, as well as the body rinses, were concentrated by means of a gentle stream of N_2, until the volume was reduced to *ca.* 20 µl. The same procedure was carried out with pure solvent to obtain blanks.

Samples were analyzed by GC–MS: *Perkin-Elmer Autosystem* gas chromatograph fitted with a *Perkin-Elmer QMass-910* mass spectrometer and a *QMass-910* analytical workstation for data analysis. The instrument was operated in scan mode at an ionization energy of 70 eV. The oven temp. was programmed for 4 min at 40°, increased at a rate of 6°/min to 280°, and maintained at this temp. for 30 min. Gas chromatograms were obtained from a *Hewlett-Packard 5890-Series-II* gas chromatograph equipped with a flame ionization detector (FID). In both cases, a 5% phenyl methyl silicone capillary column (30 m length, 0.18-mm i.d., 0.15 µm film thickness) and a split-splitless injector were used. FID and Injector were maintained at 280°. Quantification was performed by means of GC–FID detection. Identification of compounds was achieved by comparing the mass spectra with those published in the *NIST-EPA-MSDC* library or in the literature, or by using the retention-index system. Branched alkanes were identified by the presence of characteristic ions from their GC–EI-MS spectra. No authentic standards of branched alkanes were available. No derivatization of the samples was undertaken, as preliminary GC runs suggested that methylation of the extracts did not improve significantly the resolution of the chromatograms, but introduced additional contaminants that complicated data analysis.

Results. – Chemical compounds identified in the metapleural- (MG) and postpharyngeal-gland (PPG) extracts from *Solenopsis geminata* and *S. invicta* are shown in *Tables 1* and *2*, respectively, together with their chromatograms (*Fig. 1,a* and *2,a*). The MG extract of *S. invicta* contained a high proportion of fatty acid, along with Me-branched and unbranched alkanes. The MG extract of *S. geminata* also contained fatty acids and alkanes. In both species, alkaloidal compounds (6-alkyl-2-methyl-piperidines), known from their poison gland [18–21], were also found in trace amounts. Thus, we discarded the alkaloids as contaminants, because workers, when grabbed with forceps, release their poison-gland contents, which spread all over their bodies. In poison-gland extracts, four major alkyl piperidines were detected in a concentration of 8 to 37 µg per individual.

Table 1. *Concentrations* (ng/gland) *of the Compounds Identified in Metapleural* (MG) *and Postpharyngeal Glands* (PPG) of *Workers of* Solenopsis geminata *by GC-MS.* The corresponding GC signals are depicted in *Figs. 1, a* and *1, b*, resp.

Signal	Compound	MG	PPG	Signal	Compound	PPG
1	Palmitic acid	46.6	–	*22*	Heptacosane	7.8
2	Linoleic acid	69.0	–	*23*	13-Methylheptacosane	< 0.5
3	Oleic acid	29.3	–	*24*	13,15-Dimethylheptacosane	< 0.5
4	Stearic acid	10.7	–	*25*	Octacosane	5.9
5	Heneicosene	–	2.6	*26*	Nonacosene	< 0.5
6	Heneicosane	5.9	58.9	*27*	Nonacosane	12.8
7	7-, 9-, and 11-Methylhene-icosane	–	5.2	*28*	15-Methylnonacosane	< 0.5
				29	11-Methylnonacosane	< 0.5
8	Docosene	–	5.7	*30*	Triacontene	< 0.5
9	Docosane	–	10.1	*31*	Triacontane	4.8
10	Tricosene	6.4	89.7	*32*	Hentriacontene	56.8
11	Tricosane	6.1	94.2	*33*	Hentriacontane	5.6
12	9- and 11-Methyltricosane	–	3.1	*34*	15-Methylhentriacontane	< 0.5
13	Tetracosene	–	4.9	*35*	13,17-Dimethylhentriacontane	< 0.5
14	Tetracosane	1.1	5.5	*36*	Dotriacontane	1.7
15	11-Methyltetracosane	–	0.5	*37*	Tritriacontene	7.3
16	Pentacosene	–	7.2	*38*	13-, 15-, and 17-Methyltritriacontane	1.6
17	Pentacosane	2.5	13.3	*39*	13,15-Dimethyl- and 13,17-Dimethyltritriacontane	< 0.5
18	11- and 13-Methylpenta-cosane		< 0.5			
			< 0.5	*40*	Pentatriacontene	< 0.5
19	11,13-Dimethylpentacosane		< 0.5	*41*	13-Methylpentatriacontane	< 0.5
20	Hexacosane		5.9	*42*	13-Methylhexatriacontane [a])	< 0.5
21	Heptacosene		< 0.5			

[a]) Preliminary assignment.

Table 2. *Concentrations* (ng/gland) *of Compounds Identified in Metapleural* (MG) *and Postpharyngeal Glands* (PPG) *of Workers of* Solenopsis invicta *by GC-MS.* The corresponding GC signals are depicted in *Figs. 2, a* and *2, b*, resp.

Signal	Compound	MG	PPG
1	Palmitic acid	206.3	–
2	Linoleic acid	183.6	–
3	Oleic acid	206.8	–
4	Stearic acid	45.8	–
9	Docosane	10.8	–
11	Tricosane	47.1	199.8
14	Tetracosane	20.5	308.4
17	Pentacosane	34.6	485.6
20	Hexacosane	28.4	489.5
22	Heptacosane	93.0	913.4
23	13-Methylheptacosane	69.9	627.1
24	13,15-Dimethylheptacosane	36.0	488.1
23-1	3-Methylheptacosane	70.0	560.8
25	Octacosane	30.8	369.9
24-1	3,9- and 3,11-Dimethylheptacosane	54.4	363.2
31	Triacontane	34.8	65.9

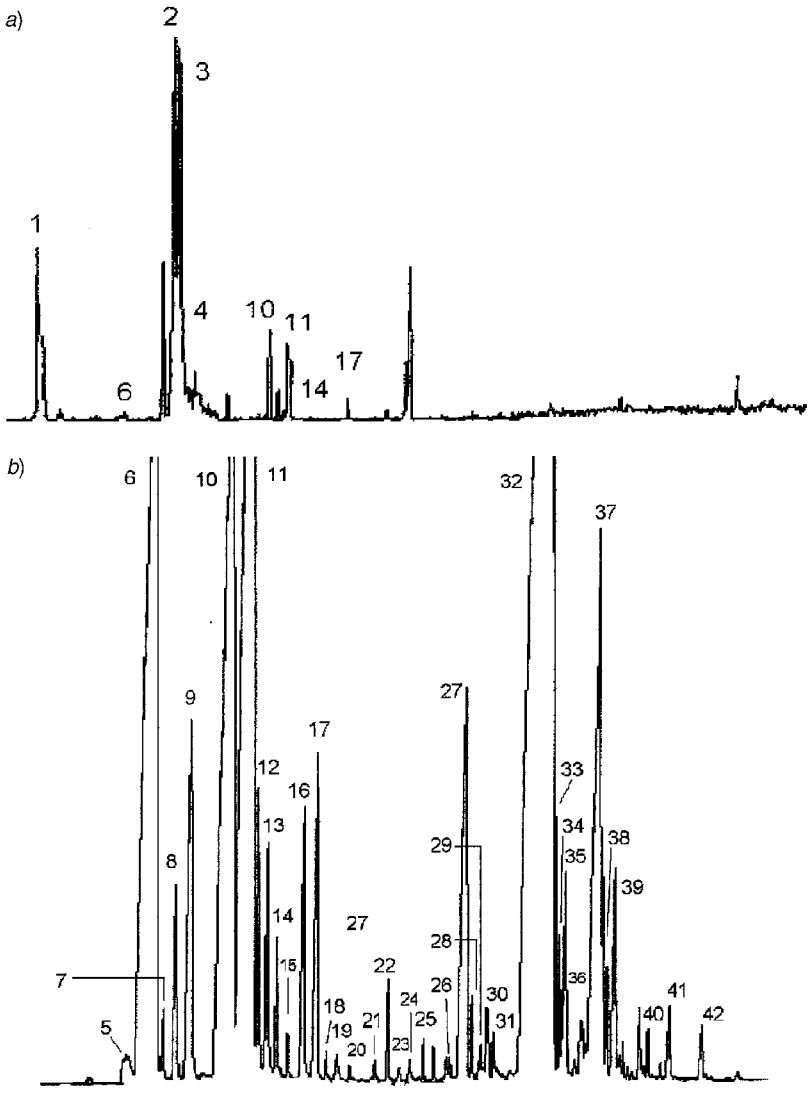

Fig. 1. *GC Chromatograms of* a) *metapleural-gland extract*; b) *postpharyngeal-gland extract*; *and* c) *body wash of* Solenopsis geminata (see *Table 1* for signal identification). Signals marked with single and double asterisks (*) are due to alkylpiperidines and cholesterol, resp.

The PPG extracts from both *Solenopsis* species contained *n*-alkanes, *n*-alkenes, and Me-branched alkanes, but *S. geminata* seems to produce a more-complex mixture of hydrocarbons (*Figs. 1,b* and *2,b*). PPG Extracts are lacking fatty acids, methyl esters, and piperidines. In each of the species of *Solenopsis*, some of the major components of the PPG extracts coincided with those present in their MG and in their cuticular-hydrocarbon samples, suggesting that basically the same hydrocarbon mixture was present in PPG, MG, and cuticle, but much more concentrated in PPG.

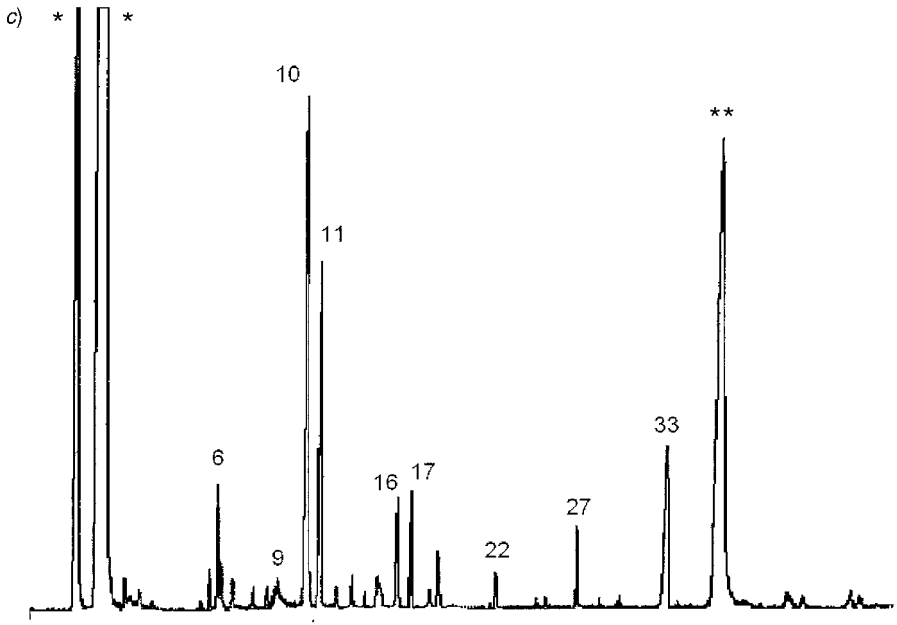

Fig. 1 (cont.)

Fatty acids were detected neither in CH_2Cl_2 body washes nor in PPG, but they were found in CH_2Cl_2 extracts of the whole body (*Figs. 1,c* and *2,c*). The total amount of these fatty acids in the whole-body extracts was 4 and 2 μg per ant for *S. invicta* and *S. geminata*, respectively.

Discussion. – Our results show that the chemical compositions of MG, PPG, and the cuticular hydrocarbons vary between the two species, despite their belonging to the same genus. The MG of these ants apparently synthesizes carboxylic acids. The concentration range of total fatty acids per individual in MG for *Solenopsis* species (0.15 μg for *S. invicta* (*Fig. 3*); 0.64 μg for *S. geminata*) is within the same range reported for the MG secretion from an individual worker of *Atta sexdens rubropilosa* (0.54–0.95 μg of total compounds), and higher than for an individual worker of *Atta cephalotes* (0.01 μg) [12].

Both species have low amounts of cuticular hydrocarbons in their MG reservoir, when compared with postpharyngeal glands, where these compounds occur in much higher concentration. *Soroker et al.* [16] demonstrated that, in *Cataglyphis niger*, PPG might function as storage of hydrocarbons, which would explain why, in several ant species, the composition of cuticular hydrocarbons is equivalent to that from the PPG content. *Bagnères* and *Morgan* [17] also compared the contents of PPG and cuticular hydrocarbons in five species of ants, and found that compositions were the same for all species. In *S. invicta*, PPG glands are more developed in queens [22], and the four major compounds identified in queens match those found in this work, *i.e.*, in *S. invicta* workers: 13-methylheptacosane, 13,15-dimethylheptacosane, 3-methylheptacosane,

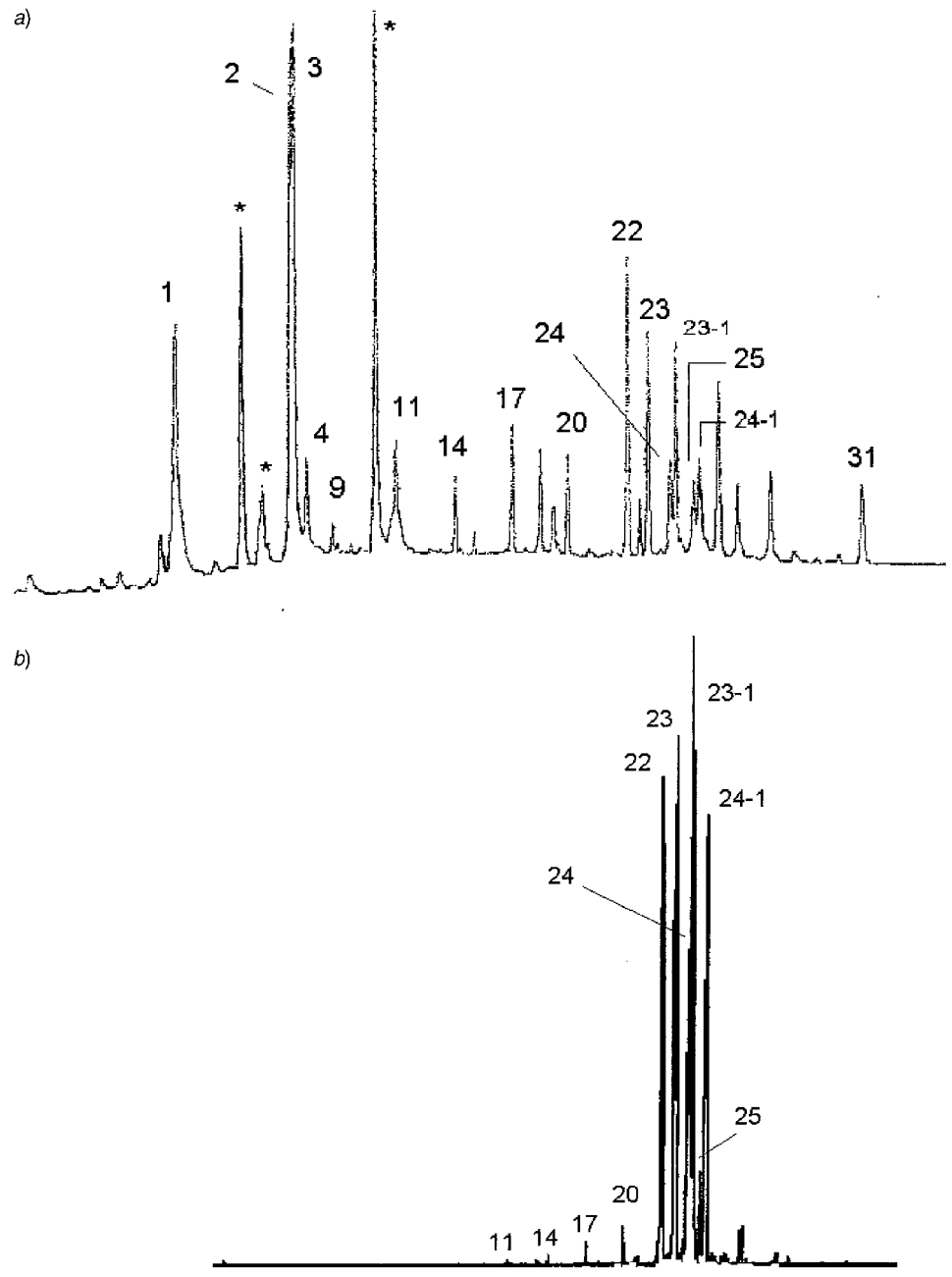

Fig. 2. *GC Chromatograms of* a) *metapleural-gland extract*; b) *postpharyngeal-gland extract*; *and* c) *body wash of* Solenopsis invicta (see *Table 2* for signal identification). Signals marked with asterisks (*) are due to alkylpiperidines.

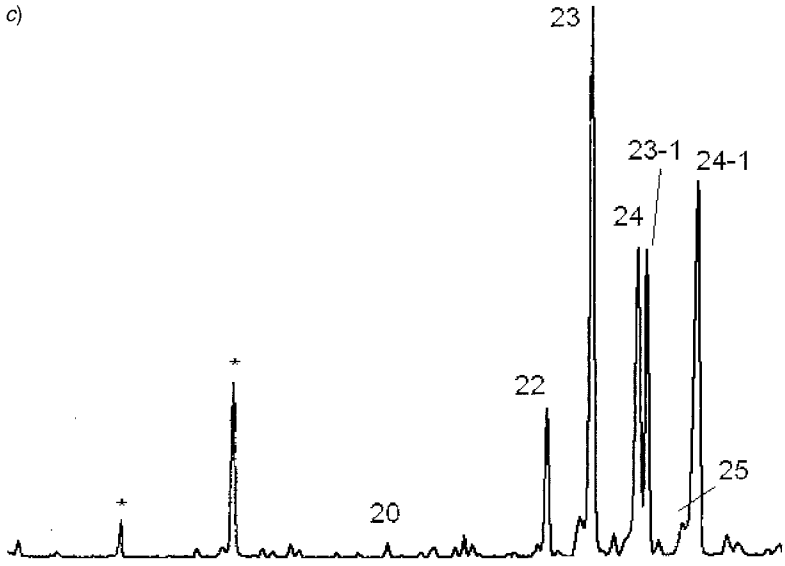

Fig. 2 (cont.)

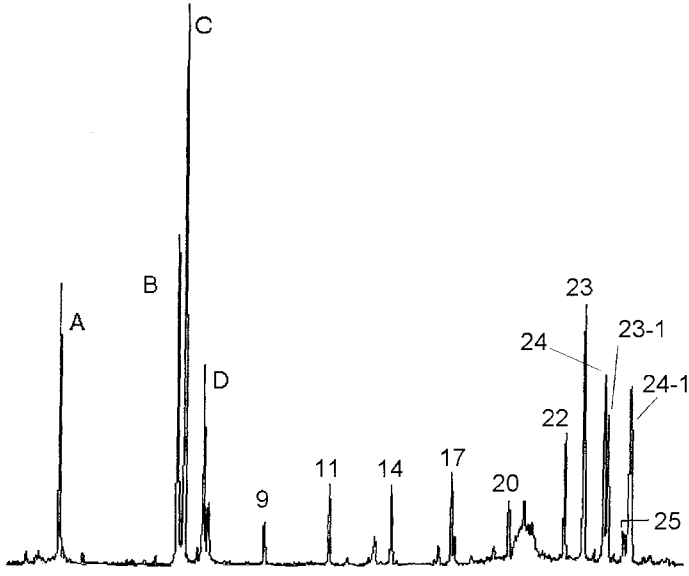

Fig. 3. *GC Chromatogram of metapleural-gland secretions of* Solenopsis invicta *recorded with a capillary tube* (see *Experimental*). For signal identification, see *Table 2*. The letters *A*, *B*, *C*, and *D* mark methyl esters of compounds of signals *1*, *2*, *3*, and *4* (*Table 2*), resp.

and a mixture of 3,9- and 3,11-dimethylheptacosane. These hydrocarbons are also present in *S. invicta* cuticular hydrocarbons extracts, as reported by *Nelson et al.* [23], and also confirmed in our analysis. In the case of *S. geminata*, hydrocarbons present in PPG extracts coincide with the compounds identified on the cuticle. There is no previous report about the chemical composition of the PPG contents of *S. geminata.* Based on this knowledge, we may rationalize the presence of cuticular hydrocarbons in MG since the MG has a permanent opening to the exterior surface of the ant's body. The substances produced in MG may, thus, spread on the substrate used by ants, but, at the same time, substances produced elsewhere may also accumulate in the reservoir of the MG, which would be the case for the cuticular hydrocarbons.

Previous studies on the behavior of workers towards body-part extracts [4] suggest that *S. geminata* uses the MG secretions as a territorial marker. No such studies are known for *S. invicta*. *Obin* and *Vander Meer* [24] proposed that, in *S. invicta*, the MG secretion is not used in nest hygiene, for which workers use the poison gland secretion instead. *S. invicta* disperses the secretion from the poison gland as an aerosol through 'gaster flagging' and, thus, these authors suggested that *S. invicta* uses the poison-gland secretion as a nest disinfectant. Here, we found that the MG reservoirs of both *S. invicta* and *S. geminata* contain fatty acids with reported antibiotic properties. These substances may, thus, be used, in addition to the poison glands, in nest, body, and territory hygiene. The carboxylic acids found in the MG secretion of the species studied are known to have antibiotic properties [6][8][10][25–27], or are repellent to arthropods [28][29]. Examples of the extensive literature about antibiotic effects of fatty acids include linoleic and oleic acid, which inhibit the growth of *Helicobacter pylori* [30], and linoleic acid, which inhibits the growth of the cyanobacterium *Phormidium tenue* [31] and of *Staphylococcus aureus* [32].

We suggest that MG secretions, when leaking onto the ground, may disinfect the ants' nests and surroundings. Once on the substrate, these substances may serve as territorial markers, as has been shown for *S. geminata* [4] and in other ant species [33]. The fatty acids and/or the hydrocarbons may serve as colony-specific signals. If cuticular hydrocarbons are used as nestmate-recognition signals, as discussed above, their extended use as territorial markers would seem plausible. Further studies of the actual dispersion of MG secretions in both species, and studies of the use of MG secretions as territorial markers in *S. invicta*, are required to answer these questions.

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