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1 AUTHOR:

Ashraf Mashaly
King Saud University

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Secretions of Dufour's gland in some ants (Hymenoptera: Formicidae)

A.M.A. Mashaly[1,2]*, A.M. Ahmed[1,2], T.M. Nunes[3] & E.D. Morgan[3]

[1]*Department of Zoology, College of Sciences, King Saud University, P.O. Box 2455,*
Riyadh, 11451, Saudi Arabia
[2]*Department of Zoology, Faculty of Science, Minia University, El Minia, Egypt*
[3]*Lennard-Jones Laboratory, Keele University, Keele, ST5 5BG, U.K.*

The ant *Pachycondyla sennaarensis* (Mayr) (Hymenoptera: Formicidae: Ponerinae) is a small dark ant, a recent accidental introduction into Saudi Arabia. It tends to be found in or near human habitations, and it is particularly unpleasant because its sting can cause severe reaction in some individuals, and there are reports of fatal effects. Analysis of the poison apparatus of *P. sennaarensis*, locally known as the samsum ant, and comparison with two other native small black ants, *Messor meridionalis* and *M. foreli* (Hymenoptera: Formicidae), have shown distinct differences in the materials in their Dufour glands and clearly distinguish *P. sennaarensis* from the two *Messor* species. *Pachycondyla sennaarensis* showed only hydrocarbons in its Dufour gland dominated by nonadecene, nonadecane and heneicosane. The powerful venom revealed no volatile compounds. The Dufour glands of *M. meridionale* and *M. foreli* had characteristic mixtures of hydrocarbons, but no venom gland volatile compounds. Pentadecane and hepadecane were the major compounds of the gland in *M. meridionalis* while nonadecane and heneicosane were the major in *M. foreli*.

Key words: Dufour gland, venom gland, pygidial gland, hydrocarbons.

INTRODUCTION

The introduction of more plants into the environment in Saudi Arabia has enlivened the surroundings, but it inevitably has brought with it unwanted effects. Many invertebrates not formerly found in the area have recently established themselves there, displacing native species (Collingwood *et al.* 1997). Imported pests can cause much damage and pose a risk to health. The problem is worse if they have no natural enemies in their new habitat. For example, the fire ants (*Solenopsis* spp.) accidentally imported into the U.S.A. from South America have proved to be a great nuisance in the southeastern part of that country, and can cause very severe stinging. *Solenopsis geminata* has also recently appeared in the Arabian Peninsula (Collingwood *et al.* 1997), but is not yet a serious problem.

Several species of the ponerine ant genus *Pachycondyla* have extremely toxic venom, causing anaphylaxis, for example *P. chinensis* (Kim *et al.* 2001; Yun *et al.* 1999; Leath *et al.* 2006), and *P. striata* (Ortiz *et al.* 2006) . One of the least pleasant of these species is *P. sennaarensis* (Mayr), commonly known as the samsum ant. First identified in West Africa, it has now established itself in the Kingdom of Saudi Arabia (Collingwood & Agosti 1996; Al-Khalifa *et al.* 2010). In Saudi Arabia it has be-

come a recognized public health hazard since its sting has resulted in some cases of fatal anaphylactic shock (Dib *et al.* 1992, 1995; Al-Shahwan *et al.* 2006; Al-Sharani *et al.* 2009). In an effort to learn more about the behaviour of this introduced ant, Mashaly *et al.* (2011) showed that workers of *P. sennaarensis* employ recruitment trail pheromones discharged from the Dufour's gland. Secretions of other abdominal glands, as well as hindgut gland secretions, did not evoke trail following.

On the other hand, the genus *Messor* is a worldwide group of seed-harvesting ants, the dominant ants in deserts and dry grasslands. *Messor meridionalis* (André) is a central Asian species extending westward into the Middle East. *Messor foreli* Santschi, is a small species and it is a true desert species common in the northern Sahara (Collingwood 1985) and in Saudi Arabia.

Despite the discovery and characterization of ant trail pheromones over the past few decades (El-Sayed 2010), surprisingly few investigations of these compounds have been undertaken for pest management. Research on the potential of using odourants in this way has targeted the control of leaf-cutting ants and the red imported fire ant (Van der Meer 1996), but the current paradigm remains

largely confined to improving the performance of toxic baits (Rust *et al.* 2004). New application technologies that deliver pheromones against invasive pest ants could help reduce our reliance on the use of insecticides for ant pest control in sensitive ecosystems or where insecticides are undesirable. Therefore, our study investigateen the secretion of some glands of *P. sennaarensis* and *M. meridionalis* as well as *M. foreli* as a first step to find chemical compounds, particularly a trail pheromone, to be tested and used in the future as modifiers for their behaviour in combination with insecticidal bait.

MATERIAL AND METHODS

All ants, the subject of this study including one ponorine ant, *P. sennaarensis*, and two myrmicine species, *M. meridionalis* and *M. foreli,* were collected from Ar Riyadh province, Kingdom of Saudi Arabia. Ant identifications were made by C.A. Collingwood, Skipton, England, and the ant specimens were deposited in his collection.

The poison and Dufour glands as well as the pygidial gland from worker ants of each species were analysed. Experiments were carried out as described in Morgan (1990). The glands were dissected in distilled water using two fine forceps under a binocular dissecting microscope (Cambridge Instruments) from the worker ants of each species in Riyadh. Each gland was separately placed in a soft glass capillary and sealed in a small flame. The sealed capillaries were transported to Keele University, U.K., where they were analysed by gas chromatography-mass spectrometry, using an Agilent 6890N Gas Chromatograph coupled to a 5973N Mass Selective detector (a quadrupole mass spectrometer using 70 eV electron impact ionization). The system was controlled by a Hewlett Packard computer with MSD ChemStation. The chromatography was carried out on a non-polar column (Hewlett Packard HP-5, 30 m × 0.25 mm with a 0.25 μm film thickness of methylsilicone) using helium as carrier gas at a constant flow rate of 1 ml/min. The samples in the sealed glass capillaries were introduced into the gas chromatograph using the solid sampling device described by Morgan (1990). The injector temperature was held at 250 °C. The samples were heated in the injector for 2 min before crushing the sealed glass capillary and beginning the chromatography. The column was initially at 50 °C, held at that temperature for

5 min, the temperature programme was started, increasing at 8 °C/min to 300 °C and holding at this temperature for 10 min. Identification was made initially by comparison of retention times with a set of standard alkanes, which covered the range found, and by mass spectrometry using the NIST 2003 computer library of mass spectra. The position of double bonds in alkenes was not determined.

RESULTS

No volatile compounds amenable to gas chromatography were identified in either the venom glands or pygidial glands of the three species. Characteristic mixtures of hydrocarbons were found in the Dufour glands of all three species. The mean values for the composition of these hydrocarbons are given in Tables 1, 2 and 3, and clearly distinguish *P. sennaarensis* from the two *Messor* species. The mixture was dominated by nonadecene, nonadecane and heneicosane in *P. sennaarensis,* that is, almost half the material was a single alkene (Table 1). Two alkanes, pentadecane and heptadecane made up more than 70 % of the contents of the gland in *M. meridionalis* (Table 2), while a more complex mixture dominated by nonadecane and heneicosene was found in the glands of *M. foreli* (Table 3). These hydrocarbon mixtures have been identified in the Dufour glands of many ant species of different subfamilies.

Mashaly *et al.* (2011) have shown that the Dufour gland is the source of the trail pheromone in *P. sennaarensis*. However, we were unable to detect

Table 1. Mean percentage composition (with standard deviations) of hydrocarbons of the Dufour glands of workers of *Pachycondyla sennaarensis* with chromatographic retention times (n = 6).

Ret. time (min)	Compound	Mean ± S.D.
11.84	Pentadecane	0.40 ± 0.48
14.40	Heptadecene	0.42 ± 0.36
14.81	Heptadecane	4.36 ± 3.32
15.74	Octadecene	0.39 ± 0.20
16.11	Octadecane	0.51 ± 0.29
16.91	Nonadecadiene	0.34 ± 0.23
17.10	Nonadecene	45.55 ± 19.61
17.45	Nonadecane	27.71 ± 2.17
18.62	Eicosane	1.26 ± 1.47
19.78	Heneicosane	17.01 ± 19.31
20.17	Not identified	0.50 ± 0.59
20.90	Docosane	1.54 ± 2.95

Table 2. Mean percentage composition (with standard deviations) of hydrocarbons of the Dufour glands of workers of *Messor meridionalis* (*n* = 6), with the chromatographic retention times.

Ret. time (min)	Compound	Mean ± S.D.
8.64	Tridecene	0.09 ± 0.12
8.73	Tridecane	8.09 ± 5.70
9.63	5-Methyltridecane	0.25 ± 0.21
9.90	3-Methyltridecane	0.39 ± 0.25
10.29	Tetradecene	0.12 ± 0.11
10.34	Tetradecane	0.47 ± 0.41
11.81	Pentadecene	7.16 ± 6.29
11.91	Pentadecane	19.09 ± 6.59
12.68	5-Methylpentadecane	0.18 ± 0.14
12.95	3-Methylpentadecane	0.83 ± 0.35
13.31	Hexadecene	0.15 ± 0.10
13.38	Hexadecane	1.78 ± 0.26
14.72	Heptadecene	8.38 ± 4.33
14.81	Heptadecane	53.02 ± 17.13

Table 3. Mean percentage composition (with standard deviations) of hydrocarbons of the Dufour glands of workers of *Messor foreli*, with the chromatographic retention times (*n* = 4).

Ret. time (min)	Compound	Mean ± S.D.
8.63	Tridecene	0.07 ± 0.05
8.76	Tridecane	5.35 ± 4.24
9.61	5-Methyltridecane	0.50 ± 0.36
9.89	3-Methyltridecane	0.55 ± 0.42
11.81	Pentadecene	8.03 ± 4.78
11.90	Pentadecane	9.65 ± 1.20
12.52	7-Methylpentadecane	0.27 ± 0.04
12.66	5-Methylpentadecane	0.49 ± 0.20
12.95	3-Methylpentadecane	1.17 ± 0.16
14.25	Not identified	0.38 ± 0.33
14.34	Hexadecadienal (?)	2.63 ± 1.29
14.72	Heptadecene	0.72 ± 0.36
14.79	Heptadecane	2.51 ± 0.18
16.14	Octadecane	1.63 ± 0.42
17.35	Nonadecene	3.29 ± 0.97
17.45	Nonadecane	38.52 ± 5.87
18.64	Eicosane	2.43 ± 1.82
19.79	Heneicosene	17.59 ± 2.31
21.72	Tricosene	3.32 ± 5.97
22.03	Tricosane	0.91 ± 0.74

any substances of higher volatility typical of trail pheromones of ants (Morgan 2008). The method used is the most sensitive available for single gland analysis, since it transfers the total contents of the gland as a single plug onto the chromatography column and is capable of detecting quantities down to about 5 ng per gland, which suggests that the amount of pheromone present is less than 5 ng per ant.

DISCUSSION

These three species of ants are readily distinguished by rapid gas chromatographic analysis of their poison apparatus. The Dufour glands of *P. sennaarensis, M. meridionalis* and *M. foreli* each contain mixtures of hydrocarbons, readily distinguished by the major compounds present. The inability to identify any volatile compounds amenable to gas chromatography in the venom glands indicated these species must all have proteinaceous venoms, which supports the finding of Orivel & Dejean (2001) that the venom of *P. sennaarensis* ant is proteinaceous.

Nikbaktzadeh *et al.* (2009a) have claimed the presence of (1,1-dimethylethyl)-2,4-bisphenol, better known as 2,4-ditert-butylphenol, in the venom of *P. sennaarensis*. This can be a contaminant, since it is not a naturally occurring substance, but is used in the manufacture of antioxidants,

and used in, for example, pharmaceuticals and fragrances. They also identified trimethylpyrazine in the venom (Nikbakhtzadeh *et al.* 2009a), a substance occasionally found in the secretions of ponerine ants, for example, in the mandibular glands of *P. obscuricornis* (Morgan *et al.* 1999), and *Diacamma ceylonense* (Morgan *et al.* 2003), but was not found in *P. sennaarensis* by us here, nor in seven other species of *Pachycondyla* (Morgan *et al.* 2003). The compound was not identified in any of the samples here. Nikbakhtzadeh *et al.* (2009a) have also reported analyses of the poison apparatus of this ant. Their results agree with those reported here, in that a similar range of hydrocarbons were reported, though not in the same proportions. They also found some oxygenated compounds, including 2-tridecyl acetate, 2-pentadecanone, dodecyl butyrate and 2-methylhexadecenal, which were not found in our analyses.

The rather different results obtained by Nikbakhtzadeh *et al.* (2009a, b) suggest that possibly samples from other areas should be analysed to find how homogeneous *P. sennaarensis* is over large areas. Neither of the other species reported here has been analysed before.

In conclusion, the Dufour glands of *P. sennaarensis*, *M. meridionalis* and *M. foreli* each contain mixtures of hydrocarbons. Again these substances will need behavioural tests in the future to clear and modify their role as a novel tactic for ant control by improving the performance of toxic baits.

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