

## COMPARATIVE SURVEY OF ABDOMINAL GLAND SECRETIONS OF THE ANT SUBFAMILY PONERINAE

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**Abstract**—The chemical contents of three abdominal glands were investigated in representative species of the ponerine ants. The Dufour glands of 14 species show a wide variety of contents. In *Myrmium camillae* and *Proceratium itoi*, no volatile substances were found in either the Dufour or venom glands. In *Ectatomma* sp., *Diacamma ceylonense*, *Diacamma indicum*, *Pachycondyla obscuricornis*, and *Pachycondyla striata*, volatile chemicals were found in the venom glands as well as in the Dufour glands. *Platythyrea punctata* was examined, but unusually it does not have a Dufour gland and its venom gland contained no volatile substances. Epoxides were found in ants for the first time in the Dufour glands of *Amblyopone reclinata*. Venom glands of *Pachycondyla tarsata* were also found to contain volatile material, including bitter-tasting cyclic dipeptides. In all, 16 species have been added to the list of those examined. All of the 27 known analyses of Dufour glands, 21 analyses of venom glands, and 4 of pygidial glands of workers of ponerine ant species have been brought together in order to seek some pattern in the type of glandular contents. Although the great majority of species produce hydrocarbons in their worker Dufour glands, and some have terpenes, there is no observable pattern for this gland on a tribe or genus level. Volatile compounds have been found in the venom glands of some

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species of the tribe Ponerini only. The information on pygidial glands is still too fragmentary for any conclusions.

**Key Words**—Hymenoptera, Formicidae, Ponerinae, Dufour gland, venom gland, pygidial gland, exocrine secretion, chemical analysis.

## INTRODUCTION

Chemical communication is important in the social organization of ants. The use of pheromones as transmitters of information allows for efficient functioning of colonies made up of tens of thousands of individuals. These pheromones serve a variety of functions, such as attracting mates, recruiting nestmates to food sources, alarm, defense, and other actions (Hölldobler and Wilson, 1990). These functions correspond to the huge variety of exocrine glands found in ants (Billen and Morgan, 1998). Pheromones with different functions originate from different glands, although one gland may have entirely different functions in separate species. The chemical nature of glandular secretions is indeed often species-specific (Blum and Hermann, 1978; Morgan et al., 1999). This fertile complexity of glands and pheromones ensures smooth social organization in the ant societies.

Ants of the subfamily Ponerinae are often considered “primitive” since they show many of the putative ancestral traits (Peeters, 1997). These comprise low queen–worker dimorphism, small colony sizes, and predatory behavior, all of which may account for simple social structures and simple communicative needs. Nevertheless, recent studies provide ample evidence that ponerine societies are more complex than previously thought (Peeters, 1997). Ponerine ants of all tribes show levels of social organization that are comparable to those in “advanced” ant subfamilies, coupled with elaborate chemical communication. Some species use complex chemical recruitment of nestmates to be able to predate other social insects (Duncan and Crewe, 1994; Hölldobler et al., 1994; Leal and Oliveira, 1995). Some even use army ant behavior in which entire colonies move along chemical trails when active (Maschwitz et al., 1989; Witte and Maschwitz, 2000). Recently, more subtle forms of social communication have been demonstrated: workers can accurately estimate levels of ovary development in their nestmates (Gobin et al., 1999; Kikuta and Tsuji, 1999), and this information seems to be contained in their cuticular hydrocarbons (Peeters et al., 1999; Liebig et al., 2000). Furthermore, these chemicals can be transferred to eggs, enabling policing of any subordinate eggs (Monnin and Peeters, 1997). Although these hydrocarbons presumably originate from abdominal exocrine glands or abdominal oenocytes, the exact glandular source remains elusive.

Because of their diversity, the Ponerinae have been systematized into eight tribes according to Bolton (1995). These are Amblyoponini (8 extant genera), Ectatommini (6 extant genera), Paraponerini (1 genus), Platythyreini (2 genera),

Ponerini (22 extant genera), Proceratiini (2 genera), and Thaumatomyrmecini and Typhlomyrmecini (1 genus each). The genera *Cerapachys* and *Cylindromyrmex* have been placed in a separate subfamily (Cerapachyinae) by Bolton (1994) but have been included in the Ponerinae by others (e.g., Hölldobler and Wilson, 1990).

Ponerine ants appear to be quite diverse in the products of their exocrine secretions. We have recently published analyses of mandibular glands of a number of ponerine species and gathered together the information on mandibular glands then available (Morgan et al., 1999). The information on abdominal exocrine glands is more fragmentary. Analyses of Dufour, poison, and pygidial glands have been made, although the information is sparse on the latter two. The pygidial gland is the putative origin of sex attractants in some species that show sexual calling behavior (i.e., lifting the gaster and exposing intersegmental membranes), but the precise origin and chemistry have never been elucidated (Hölldobler and Haskins, 1977). The position is similar with tandem running. While its existence is recognized and its nature is chemical, the identification and origin of the pheromone remains unknown, although Hölldobler and Traniello (1980) showed that the pygidial gland secretion is necessary to cause *Pachycondyla obscuricornis* to perform tandem running behind a dummy.

With our study, we aim to fill the gap between increasing biological evidence for complex chemical communication and the source of the putative pheromones. We have attempted to obtain at least one species from each of the six common tribes in the system of Bolton (1995), excluding only the two monogeneric and cryptic tribes Thaumatomyrmecini and Typhlomyrmecini. We expand and review the general knowledge of the chemical nature of abdominal gland secretions in the Ponerinae and show the diversity of chemical components within and across genera. The diversity of compounds documented will allow testing of various behavioral functions that were previously attributed to chemistry, without pheromonal identification. Our data should permit quick testing of putative pheromones in functional studies.

#### METHODS AND MATERIALS

In general, foraging workers were collected for dissection and analysis (Table 1). Dufour glands, pygidial glands, and poison glands were dissected separately, and the dissected glands immediately sealed in thin-walled glass capillaries according to the solid-sampling technique (Morgan and Wadhams, 1972; Morgan, 1990).

Gas chromatography–mass spectrometry (GC-MS) was carried out on an Hewlett Packard 5890 gas chromatograph and 5970 mass selective detector quadrupole mass spectrometer with 70 eV ionization. Chromatography was carried out on a fused silica capillary column (12 m × 0.32 mm with a 0.3- $\mu$ m coating of

TABLE 1. PONERINE SPECIES STUDIED AND COLLECTION SITES

| Tribe         | Species, authority                               | Location, Country <sup>a</sup>            |
|---------------|--|---|
| Amblyoponini  | <i>Amblyopone reclinata</i> Mayr, 1879           | Bogor                                     |
|               | <i>Mystrium camillae</i> Emery, 1889             | Kebun Raya, Bogor, Indonesia              |
| Ectatommini   | <i>Ectatomma</i> sp.                             | Maceió, AL, Brazil                        |
|               | <i>Gnamptogenys menadensis</i> Mayr, 1887        | Karaenta, Sulawesi, Indonesia             |
| Platythyreini | <i>Platythyrea cribinodis</i> (Gerstäcker, 1859) | Hunter's Lodge, Nairobi, Kenya            |
| Ponerini      | <i>Platythyrea punctata</i> Smith, 1858          | Florida, USA                              |
|               | <i>Diacamma celonense</i> Emery, 1897            | Masinagudi, TN, India                     |
|               | <i>Diacamma indicum</i> (Santschi, 1920)         | Masinagudi, TN, India                     |
|               | <i>Dinoponera australis</i> Emery, 1901          | Itirapina, SP, Brazil                     |
|               | <i>Odontomachus bauri</i> Emery, 1892            | Maceió, AL, Brazil                        |
|               | <i>Odontoponera transversa</i> (F. Smith, 1857)  | Singapore                                 |
|               | <i>Pachycondyla indica</i> (Emery, 1899)         | Bangalore, KT, India                      |
|               | <i>Pachycondyla obscuricornis</i> (Emery, 1890)  | Viçosa, MG, Brazil                        |
|               | <i>Pachycondyla striata</i> (Santschi, 1930)     | Rio Claro, SP, Brazil                     |
| Proceratiini  | <i>Pachycondyla tarsata</i> (Fabricius, 1789)    | Ellisras, Northern Province, South Africa |
|               | <i>Proceratium itoi</i> (Forel, 1918)            | Takamatsu, Japan                          |

<sup>a</sup>AL = Alagoas, KT = Karnataka, MG = Minas Gerais, SP = São Paulo, TN = Tamil Nadu.

polydimethylsiloxane; SGE, Milton Keynes, UK). Samples were heated in the injector to 150°C before crushing in a Keele solid-sample injector (Morgan, 1990). The oven was programmed from 30°C to 150°C at 8°C/min and then held isothermally. The carrier gas was helium at 1 ml/min.

Mass spectral identifications were confirmed wherever possible by comparison of retention times and mass spectra with those of synthetic standards. Where pure standards were not available, identification was based on comparison with spectra in our own collection and in computer libraries (NIST Library, 2001).

## RESULTS

The species of Ponerinae examined in this study and their place of collection are listed in Table 1.

*Amblyoponini*. Two examples of the tribe Amblyoponini were available. *Amblyopone reclinata* had a simple mixture of four related epoxides in its Dufour glands, their structures were confirmed with the help of mass determinations on the molecular ions and the principal fragment ions. The first substance, 9,10-epoxyoctadecane ( $M^+$  250) formed only 0.5% of the total, the second,

9,10-epoxy-6-nonadecene ( $M^+$  280) was 0.9%. The third, the major component, *cis*-9,10-epoxynonadecane ( $M^+$  282) ( $m/z$  264, 253, 239, 225, 211, 197, 183 (all weak), 169 (26%), 155 (30), 138 (10), 124 (15), 109 (12), 97 (39), 83 (82), 96 (92), 69 (92), 55 (100), 43 (95), 41 (92), represented over 98% of the total (the results are, therefore, not tabulated). The fourth compound, *trans*-9,10-epoxynonadecane ( $M^+$  282) is less than 0.05% of the total. This is the first report of epoxides in the exocrine glands of ants. We found no volatiles in either the Dufour or venom glands of *Mystridium camillae*. There have been earlier examples of species without volatile substances in the Dufour glands, but it is not a frequent phenomenon.

*Ectatommini*. The *Ectatomma* species from Brazil, probably a new species, had a simple mixture of hydrocarbons in its Dufour glands, with nonadecene the major component (Table 2). Examination of the venom glands revealed no volatile compounds. In *Gnamptogenys menadensis*, we found a group of unidentified  $\delta$ -lactones and methyl 6-methylsalicylate. The sticky and smelly hydrophobic mixture of these compounds was apparent each time the ants were handled with forceps.

*Platythyreini*. One unidentified species from Kenya contained heptadecene with small amounts of related alkenes and alkanes (Table 2). The venom glands were empty of volatile components. *Platythyrea punctata* from Florida was also

TABLE 2. COMPOSITION OF DUFOUR GLANDS OF *Ectatomma* SP.( $N = 10$ ) AND *Platythyrea cribinodis* WORKERS

| Component         | Compound       | Method of identification | Proportion (% , mean $\pm$ SD) |                      |
|-------------------|----------------|--------------------------|--------------------------------|----------------------|
|                   |                |                          | <i>Ectatomma</i> sp.           | <i>P. cribinodis</i> |
| 1                 | Pentadecene    | RT, MS                   |                                | 0.67 $\pm$ 0.95      |
| 2                 | Pentadecane    | RT, MS                   |                                | 2.98 $\pm$ 2.01      |
| 3                 | Hexadecene     | RT, MS                   |                                | 1.19 $\pm$ 0.59      |
| 4                 | Hexadecane     | RT, MS                   |                                | 0.18 $\pm$ 0.25      |
| 5                 | Heptadecadiene | MS                       |                                | 12.35 $\pm$ 0.15     |
| 6                 | Heptadecene    | MS                       | 4.4 $\pm$ 2.6                  | 55.23 $\pm$ 4.54     |
| 7                 | Heptadecane    | RT, MS                   | 1.7 $\pm$ 1.4                  | 3.00 $\pm$ 0.53      |
| 8                 | Octadecene     | MS                       | 2.4 $\pm$ 1.8                  | 0.22 $\pm$ 0.30      |
| 9                 | Nonadecadiene  | MS                       | 3.1 $\pm$ 2.2                  | 2.11 $\pm$ 0.27      |
| 10                | Nonadecene     | MS                       | 88.9 $\pm$ 6.9                 | 11.02 $\pm$ 2.39     |
| 11                | Nonadecane     | RT, MS                   | <0.1                           | 0.25 $\pm$ 0.35      |
| 12                | Heneicosene    | MS                       |                                | 4.91 $\pm$ 0.42      |
| 13                | Heneicosane    | RT, MS                   |                                | 0.39 $\pm$ 0.55      |
| 14                | Tricosene      | RT, MS                   |                                | 0.96 $\pm$ 0.44      |
| 15                | Tricosane      | RT, MS                   |                                | 3.31 $\pm$ 0.75      |
| 16                | Pentacosane    | RT                       |                                | 0.70 $\pm$ 0.81      |
| 17                | Heptacosane    | RT                       |                                | 0.57 $\pm$ 0.81      |
| Mean total amount |                |                          | 1.4 $\pm$ 1.3 $\mu$ g          | 230 ng               |

TABLE 3. COMPOSITION OF DUFOUR GLANDS OF *Diacamma ceylonense* WORKERS ( $N = 15$ ) AND *D. indicum* ( $N = 4$ )

| Component              | Compound            | Method of identification | Proportion (% , mean $\pm$ SD) |                   |
|------------------------|---------------------|--------------------------|--------------------------------|-------------------|
|                        |                     |                          | <i>D. ceylonense</i>           | <i>D. indicum</i> |
| 1                      | Tridecane           | RT, MS                   |                                | 0.9 $\pm$ 0.3     |
| 2                      | Tetradecane         | RT, MS                   | 0.8 $\pm$ 0.2                  | 1.1 $\pm$ 0.5     |
| 3                      | 7-Methyltetradecane | MS                       | 0.6 $\pm$ 0.2                  | 1.4 $\pm$ 0.8     |
| 4                      | Pentadecadiene      | MS                       | 1.1 $\pm$ 0.5                  | 2.7 $\pm$ 3.4     |
| 5                      | Pentadecene         | MS                       | 8.0 $\pm$ 1.6                  | 8.7 $\pm$ 4.3     |
| 6                      | Pentadecane         | RT, MS                   | 11.9 $\pm$ 3.1                 | 16.6 $\pm$ 3.7    |
| 7                      | Hexadecene          | MS                       | 0.6 $\pm$ 0.4                  | 0.8 $\pm$ 0.9     |
| 8                      | Hexadecane          | RT, MS                   | 1.8 $\pm$ 0.2                  | 4.3 $\pm$ 7.8     |
| 9                      | Heptadecadiene      | MS                       | 7.4 $\pm$ 2.0                  | 7.1 $\pm$ 0.6     |
| 10                     | Heptadecene         | MS                       | 30.7 $\pm$ 4.8                 | 21.2 $\pm$ 8.7    |
| 11                     | Heptadecane         | RT, MS                   | 1.6 $\pm$ 0.3                  | 2.9 $\pm$ 1.1     |
| 12                     | Octadecene          | MS                       | 0.3                            | 0.8 $\pm$ 0.7     |
| 13                     | Octadecane          | RT, MS                   | 0.4 $\pm$ 0.1                  | 0.9 $\pm$ 0.5     |
| 14                     | Nonadecadiene       | MS                       | 1.6 $\pm$ 0.5                  | 0.5 $\pm$ 0.4     |
| 15                     | Nonadecene          | MS                       | 2.8 $\pm$ 0.6                  | 3.6 $\pm$ 0.7     |
| 16                     | Nonadecane          | RT, MS                   | 4.5 $\pm$ 1.1                  | 4.5 $\pm$ 1.2     |
| 17                     | Eicosane            | RT, MS                   | 1.6 $\pm$ 0.5                  | 1.3 $\pm$ 0.3     |
| 18                     | Heneicosene         | MS                       | 3.0 $\pm$ 0.8                  | 2.1 $\pm$ 6.2     |
| 19                     | Heneicosane         | RT, MS                   | 5.6 $\pm$ 0.7                  | 4.4 $\pm$ 0.3     |
| 20                     | Tricosene           | RT, MS                   | 2.6 $\pm$ 0.4                  | 2.1 $\pm$ 0.1     |
| 21                     | Tricosane           | RT, MS                   | 1.4 $\pm$ 1.0                  | 0.9 $\pm$ 0.4     |
| 22                     | Tetracosene         | MS                       | 0.4 $\pm$ 0.5                  | 0.4 $\pm$ 1.3     |
| 23                     | Pentacosene         | MS                       | 4.1 $\pm$ 6.4                  | 3.3 $\pm$ 1.0     |
| 24                     | Pentacosane         | RT, MS                   | 2.0 $\pm$ 0.9                  | 2.1 $\pm$ 1.3     |
| 25                     | Hexacosene          | MS                       | 0.5 $\pm$ 0.5                  | 0.2               |
| 26                     | Hexacosane          | RT, MS                   | 0.3 $\pm$ 0.4                  | 1.0 $\pm$ 0.4     |
| 27                     | Heptacosene         | MS                       | 4.4 $\pm$ 5.9                  | 4.2 $\pm$ 1.1     |
| Mean total amount (ng) |                     |                          | 992                            | 1013              |

examined, but this species is unusual in that it does not possess a Dufour gland and its venom glands and sternal glands did not contain any volatile material.

*Ponerini*. The Dufour glands of the two species investigated had similar mixtures of linear alkenes and alkanes. *Diacamma ceylonense* had heptadecene as the major compound, and *Diacamma indicum* from India had almost equal proportions of pentadecane and heptadecene (Table 3). No oxygenated compounds or terpenes were detected. The venom glands contained small but significant amounts of volatiles, dominated in both by up to 200 ng of 2-undecanone but averaging less than that (Table 4).

Distinct differences were found between the workers and a single sample of the gamergate of *Dinoponera australis* from a single nest. The workers had

TABLE 4. COMPOSITION OF VENOM GLAND VOLATILE SUBSTANCES OF *Diacamma ceylonense* (N = 13) AND *D. indicum* (N = 3) WORKERS<sup>a</sup>

| Component         | Compound                | Mean proportion (%)  |                   |
|-------------------|-------------------------|----------------------|-------------------|
|                   |                         | <i>D. ceylonense</i> | <i>D. indicum</i> |
| 1                 | Methyl butanoate        | 2.07                 | 21.60             |
| 2                 | 2-Heptanone             |                      | 1.30              |
| 3                 | Heptanoic acid          | 0.57                 | 6.80              |
| 4                 | 2,4,6-Trimethylpyrazine |                      | 3.53              |
| 5                 | 2-Undecanone            | 96.7                 | 60.63             |
| 6                 | Indole                  | 0.63                 | 6.10              |
| Total amount (ng) |                         | 42.2                 | 29.6              |

<sup>a</sup>All compounds were identified by mass spectra and comparison of retention times with authentic samples.

a complex mixture of up to 60 components, with the hydrocarbons nonadecene, heneicosene, octadecene, heptadecene, and heptadecadiene, in decreasing order, as the most abundant. Nonadecene was by far the most abundant. There was considerably less of the hydrocarbons in the gamergate, but nonadecene, heneicosene, and heneicosadiene were the important ones. Both workers and gamergate contained docosanol, the proportion of the alcohol being much greater in the gamergate. These results have not been tabulated because the amounts varied considerably among individuals in the one colony, and only one gamergate was analyzed. Attempts to obtain further samples of this species were unsuccessful.

The Dufour gland of *Odontomachus bauri* contained a relatively simple mixture of hydrocarbons with 8-heptadecene the major one, closely followed by heptadecadiene (Table 5). There were also three acyclic diterpenes. The first was a compound first identified in the myrmeciine ant *Myrmecia nigriceps* and there called T<sub>1</sub> (Jackson et al., 1989). It had a retention index of 1770, a molecular mass of 262, and a mass spectrum similar to that of geranylgeraniol. Its molecular formula appears to be C<sub>19</sub>H<sub>34</sub>, and is, therefore, a partly reduced and degraded diterpene. It has also been found in *Pachycondyla apicalis* (Cruz Lopez and Morgan, 1997), and in this work also in *Odontoponera transversa* and *Pachycondyla obscuricornis*. The second compound has a similar mass spectrum to the first (base peak at *m/z* 69, but with a strong ion at *m/z* 95, 30% intensity) and a molecular mass of 278. Its molecular formula is apparently C<sub>20</sub>H<sub>38</sub>. This compound was also found in *Pachycondyla obscuricornis*. The third diterpene (probable molecular mass 294, mass spectrum similar to the second, suggested molecular formula C<sub>20</sub>H<sub>38</sub>O) was also found in *Pachycondyla apicalis*. and *P. obscuricornis*. This third compound is thought to be an alcohol derivative of the second compound. The venom glands were examined but no volatile substances were found.

TABLE 5. COMPOSITION OF DUFOUR GLAND SECRETION OF *Odontomachus bauri* WORKERS ( $N = 10$ )

| Component                    | Compound  | Method of identification | Proportion (% , mean $\pm$ SD) |
|------------------------------|---|--------------------------|--------------------------------|
| 1                            | Tetradecane                                       | RT, MS                   | 0.7 $\pm$ 0.3                  |
| 2                            | Pentadecane                                       | RT, MS                   | 2.9 $\pm$ 0.9                  |
| 3                            | Pentadecane                                       | RT, MS                   | 15.1 $\pm$ 2.9                 |
| 4                            | Hexadecadiene                                     | MS                       | 1.2 $\pm$ 0.2                  |
| 5                            | Hexadecene  | RT, MS                   | 2.0 $\pm$ 0.4                  |
| 6                            | Hexadecane  | RT, MS                   | 0.7 $\pm$ 0.4                  |
| 7                            | Heptadecadiene                                    | MS                       | 26.0 $\pm$ 2.9                 |
| 8                            | 8-Heptadecene                                     | RT, MS                   | 32.9 $\pm$ 3.5                 |
| 9                            | Heptadecane                                       | RT, MS                   | 2.7 $\pm$ 0.8                  |
| 10                           | Acyclic terpene C <sub>19</sub> H <sub>34</sub>   | MS                       | 5.1 $\pm$ 2.8                  |
| 11                           | Geranylgeraniol                                   | MS                       | 9.1 $\pm$ 6.4                  |
| 12                           | Acyclic terpene C <sub>20</sub> H <sub>38</sub> O | MS                       | 1.1 $\pm$ 1.2                  |
| Mean total amount ( $\mu$ g) |   |                          | 1.47 $\pm$ 1.25                |

The Dufour glands of *Odontoponera transversa* contained a mixture of alkanes; branched alkanes and alkenes; the linear terpene C<sub>19</sub>H<sub>34</sub> also found in *Odontomachus bauri*, *Pachycondyla apicalis*, and *Pachycondyla obscuricornis*; and a small amount of geranylgeraniol. The most abundant substance was pentadecane (Table 6). Geranylgeraniol has also been found in Dufour glands of *Ectatomma ruidum* (Bestmann et al., 1995) as well as in the doryline ant *Aenictus rotundatus* (Oldham et al., 1994) and the formicine ants *Formica fusca*, *F. nigricans*, and *F. polyctena* (Bergström and Löfqvist, 1973), and in the labial glands of several species of bumblebees.

Four species of *Pachycondyla* (*P. indica*, *P. obscuricornis*, *P. striata*, and *P. tarsatus*) have been added to the one species (*P. apicalis*) from Mexico already examined (Cruz Lopez and Morgan, 1997). *P. indica* contained a complex mixture of linear and methyl-branched hydrocarbons, with pentadecane dominant (Table 7). *P. striata* contained a complex mixture of hydrocarbons, with heptadecane most abundant, but with five unidentified sesterterpenes (C<sub>25</sub> compounds) (Table 7) with mass spectra similar to, but not the same as, that of geranylarnesol found in the Dufour gland of the stingless bee *Frieseomelitta languida* (Cruz López et al., in preparation). Such compounds are unusual and need further chemical examination. *P. obscuricornis* contained no linear hydrocarbons but a mixture of two groups of diterpene homolog, one group of four compounds with base peak at  $m/z$  69, exemplified by the substance C<sub>19</sub>H<sub>34</sub> of mass 262 (Table 8), also found in *Myrmecia nigriceps*, *Pachycondyla apicalis*, *Odontomachus bauri*, and *Odontoponera transversa*. The second and third of this series were the same as the second and third in *Odontomachus bauri*. The second series of four diterpenes apparently

TABLE 6. COMPOSITION OF THE SECRETION OF DUFOUR GLANDS OF WORKERS OF *Odontoponera transversa* ( $N = 10$ )

| Component                    | Compound  | Method of identification | Proportion (% , mean $\pm$ SD) |
|------------------------------|---|--------------------------|--------------------------------|
| 1                            | Tridecane                                       | RT, MS                   | 2.3 $\pm$ 1.4                  |
| 2                            | 3-Methyltridecane                               | MS                       | 0.04 $\pm$ 0.09                |
| 3                            | Tetradecane                                     | RT, MS                   | 3.3 $\pm$ 1.5                  |
| 4                            | 3-Methyltetradecane                             | MS                       | 0.08 $\pm$ 0.2                 |
| 5                            | Pentadecadiene                                  | MS                       | 0.2 $\pm$ 0.2                  |
| 6                            | Pentadecene                                     | RT, MS                   | 3.2 $\pm$ 1.1                  |
| 7                            | Pentadecane                                     | RT, MS                   | 38.0 $\pm$ 7.4                 |
| 8                            | 7-Methylpentadecane                             | MS                       | 0.01 $\pm$ 0.02                |
| 9                            | 5-Methylpentadecane                             | MS                       | 0.03 $\pm$ 0.05                |
| 10                           | Hexadecadiene                                   | MS                       | 1.4 $\pm$ 0.4                  |
| 11                           | Hexadecene                                      | RT, MS                   | 1.4 $\pm$ 0.2                  |
| 12                           | Hexadecane                                      | RT, MS                   | 1.2 $\pm$ 0.2                  |
| 13                           | Heptadecadiene                                  | MS                       | 19.5 $\pm$ 5.6                 |
| 14                           | Heptadecene                                     | MS                       | 19.6 $\pm$ 3.9                 |
| 15                           | Heptadecane                                     | RT, MS                   | 4.0 $\pm$ 1.3                  |
| 16                           | Nonadecadiene                                   | MS                       | 0.4 $\pm$ 0.7                  |
| 17                           | Nonadecene                                      | MS                       | 1.9 $\pm$ 2.1                  |
| 18                           | Nonadecane                                      | RT, MS                   | 0.6 $\pm$ 1.1                  |
| 19                           | Heneicosene                                     | MS                       | 0.4 $\pm$ 0.5                  |
| 20                           | Tricosene                                       | RT, MS                   | 0.4 $\pm$ 0.4                  |
| 21                           | Acyclic terpene C <sub>19</sub> H <sub>34</sub> | MS                       | 1.0 $\pm$ 0.5                  |
| 22                           | Geranylgeraniol                                 | MS                       | 0.8 $\pm$ 1.0                  |
| 23                           | Geranylcitronellol (?)                          | MS                       | 0.2 $\pm$ 0.1                  |
| Mean total amount ( $\mu$ g) |   |                          | 29.4 $\pm$ 29.8                |

contained a homomevalonate unit since their mass spectra showed strong ions at  $m/z$  55 and 83 instead of  $m/z$  41 and 69. These substances are all derived from acyclic diterpenes, some of them having lost one carbon atom, but their structures remain unsolved. The venom glands of *P. tarsatus* were also examined. The samples were divided into those in which the venom had a bitter taste and those that were not bitter. The samples were variable in their content, but most contained a rich mixture of nitrogenous compounds. The greatest part consisted of dimethylaminoethanol, 2,6-dimethylpiperidine (two isomers, presumably *cis* and *trans*), indole, cyclopropylleucine, and a compound with a mass spectrum corresponding closely to that of *N*-2-methylbutyl-*N*-2-methylbutylideneamine (otherwise, the aldimine or Schiff's base produced from 2-methylbutylamine and 2-methylbutanal). The 11 samples of bitter-tasting venom were variable, but 8 of them contained, in addition to cyclo-leucylproline (1,4-diaza-2,5-dioxo-3-isobutylbicyclo[4.3.0]nonane) smaller amounts of cycloglycylproline 1,4-diaza-2,5-dioxobicyclo[4.3.0]nonane, and cycloalanylleucine.

TABLE 7. COMPOSITION OF DUFOUR GLAND VOLATILE SUBSTANCES OF *Pachycondyla striata* (N = 5) AND *P. indica* (N = 2) WORKERS

| Component                    | Compound  | Method of identification | Proportion (%; mean $\pm$ SD) |                  |
|------------------------------|---|--------------------------|-------------------------------|------------------|
|                              |   |                          | <i>P. striatas</i>            | <i>P. indica</i> |
| 1                            | Limonene  | RT, MS                   | 0.7                           |                  |
| 2                            | 2,5-Dimethyl-3-butylpyrazine                      | RT, MS                   | 1.3                           |                  |
| 3                            | Tridecene   | MS                       |                               | 0.2              |
| 4                            | Tridecane   | RT, MS                   |                               | 8.6              |
| 5                            | 7-Methyltridecane                                 | MS                       |                               | 0.2              |
| 6                            | 3-Methyltridecane                                 | MS                       |                               | 0.7              |
| 7                            | Tetradecane                                       | RT, MS                   |                               | 4.0              |
| 8                            | 7-Methyltetradecane                               | MS                       | 0.6                           | 2.8              |
| 9                            | 5-Methyltetradecane                               | MS                       |                               | 4.6              |
| 10                           | 3-Methyltetradecane                               | MS                       |                               | 1.8              |
| 11                           | Pentadecene                                       | RT, MS                   |                               | 0.1              |
| 12                           | Pentadecane                                       | RT, MS                   | 4.9                           | 38.0             |
| 13                           | 12-Methylpentadecane                              | MS                       | 1.0                           |                  |
| 14                           | 7-Methylpentadecane                               | MS                       | 0.5                           |                  |
| 15                           | 7-Methylpentadecane                               | MS                       |                               | 0.6              |
| 16                           | Hexadecene  | MS                       | 0.3                           | 0.1              |
| 17                           | Hexadecane  | RT, MS                   | 2.9                           | 2.2              |
| 18                           | Heptadecadiene                                    | MS                       | 1.9                           | 1.7              |
| 19                           | Heptadecene                                       | RT, MS                   | 5.3                           | 7.0              |
| 20                           | Heptadecane                                       | RT, MS                   | 34.6                          | 11.2             |
| 21                           | Octadecadiene                                     | MS                       | 1.3                           |                  |
| 22                           | Octadecene  | MS                       | 3.4                           |                  |
| 23                           | Octadecane  | RT, MS                   | 0.5                           |                  |
| 24                           | Acyclic terpene C <sub>19</sub> H <sub>34</sub>   | MS                       | 2.3                           | 0.5              |
| 25                           | Nonadecadiene                                     | MS                       | 1.0                           |                  |
| 26                           | Nonadecene  | RT, MS                   | 11.9                          | 2.5              |
| 27                           | Nonadecane  | RT, MS                   | 0.5                           | 2.0              |
| 28                           | Heneicosene                                       | MS                       |                               | 0.5              |
| 29                           | Heneicosane                                       | MS                       |                               | 0.3              |
| 30                           | Tricosene   | RT, MS                   |                               | 7.8              |
| 31                           | Tricosane   | RT, MS                   |                               | 2.3              |
| 32                           | Acyclic terpene C <sub>19</sub> H <sub>36</sub> O | MS                       | 1.6                           |                  |
| 33                           | Terpene 2   | MS                       | 24.3                          |                  |
| 34                           | Terpene 3   | MS                       | 0.6                           |                  |
| 35                           | Terpene 4   | MS                       | 1.1                           |                  |
| 36                           | Terpene 5   | MS                       | 1.7                           |                  |
| Mean total amount ( $\mu$ g) |   |                          | 1.8                           | 1.0              |

*Proceratiini*. We found no volatile substances in the Dufour glands, venom glands, or pygidial glands of *Proceratium itoi*. This was the second species in this work without volatile material in the Dufour glands.

TABLE 8. COMPOSITION OF DUFOUR GLAND VOLATILE SUBSTANCES OF *Pachycondyla obscuricornis* (N = 3) WORKERS<sup>a</sup>

| Component              | Retention time (min) | Mass spectral ions (with relative intensity, %) <sup>b</sup>   | Proportion (%; mean ± SD) |
|------------------------|----------------------|--|---------------------------|
| 1                      | 19.67                | M <sup>+</sup> 280, 195, 193, 178, 151, 137, 136, 123, 121, 109, 95, 81 (30), 69 (100), 55, 41 (35)                              | 0.9                       |
| 2                      | 20.19                | M <sup>+</sup> 294, m/z 207, 150, 137, 123, 109, 95 (30), 83, 81, 69 (100), 55, 41 (35)  | 0.6                       |
| 3                      | 20.5                 | M <sup>+</sup> 294, m/z 247, 233, 207, 192, 150, 137, 123, 109, 95 (45), 81, 69 (100), 55, 41 (45)                               | 17.4                      |
| 4                      | 20.64                |  | 0.5                       |
| 5                      | 20.7                 | M <sup>+</sup> 294, m/z 219, 193, 150, 137, 125, 109, 95 (25), 83 (80), 81 (35), 69 (40), 55 (100), 41 (40)                      | 5.5                       |
| 6                      | 20.8                 |  | 0.9                       |
| 7                      | 21.3                 | M <sup>+</sup> 294, 261, 221, 219, 151, 137, 123, 109, 95 (55), 83, 81, 69 (100), 55, 41 (55)                                    | 1.6                       |
| 8                      | 21.58                | M <sup>+</sup> 294, 261, 233, 207, 191, 177, 164, 151, 135, 123, 109 (20), 95 (55), 83 (65), 69 (40), 67 (30), 55 (100), 41 (50) | 67.2                      |
| 9                      | 21.68                | M <sup>+</sup> 294, 259, 231, 205, 177, 149, 135, 123, 121, 109 (25), 95 (35), 83 (60), 69, 67 (35), 55 (100), 41 (45)           | 1.9                       |
| 10                     | 22.3                 | (M <sup>+</sup> 298 ?), 275, 247, 233, 221, 165, 151, 135, 123, 109 (25), 95 (50), 83 (70), 69 (35), 67 (32), 55 (100), 41 (45)  | 3.2                       |
| 11                     | 22.5                 |  | 0.4                       |
| Mean total amount (μg) |                      |  | 1.2                       |

<sup>a</sup>The secretion consisted of unidentified terpenes, so their relative retention times and principal mass spectral ions are given.

<sup>b</sup>No intensity recorded means a weak ion.

DISCUSSION

The tribe Amblyoponini contains eight living genera, *Adetomyrma*, *Amblyopone*, *Concoctio*, *Myopone*, *Mystrium*, *Onychomyrmex*, *Prionopelta*, and *Parapriopelta*. Table 9 brings together our work here with earlier reports on Dufour glands. One species of *Amblyopone* has hydrocarbons like many other ponerines, but *A. reclinata* introduces a previously unencountered theme of essentially one substance, an epoxide. *Mystrium camillae* contained no volatile substances in Dufour or venom glands and shares this trait with one other species. Table 10 summarizes our knowledge on ponerine venom glands. The information here is fragmentary. It is not common to find volatile materials in venom glands of ants, and there has been little study of their protein venoms. Table 11, which summarizes our knowledge of pygidial glands, contains even less information.

TABLE 9. SUMMARY OF RESULTS OF INVESTIGATIONS OF DUFOUR GLANDS OF PONERINE ANTS

| Tribe, genus, species                          | Chemicals  | Reference                                 |
|--|--|---|
| Amblyoponini                                   |  |   |
| <i>Amblyopone australis</i>                    | Hydrocarbons   | Cavill and Williams (1967)                |
| <i>Amblyopone reclinata</i>                    | 9,10-Epoxy-nonadecane  | This work                                 |
| <i>Mystrium camillae</i>                       | Nothing volatile   | This work                                 |
| Ectatommini                                    |  |   |
| <i>Ectatomma ruidum</i>                        | Geranylgeranyl acetate and geranylgeraniol   | Bestmann et al. (1995)                    |
| <i>Ectatomma</i> sp.                           | C <sub>17</sub> –C <sub>19</sub> alkanes and alkenes, about 90% nonadecene   | This work                                 |
| <i>Gnamptogenys menadensis</i>                 | C <sub>13</sub> –C <sub>19</sub> alkanes and alkenes and farnesenes  | Gobin et al. (1998)                       |
| <i>Rhytidoponera aciculata</i> <sup>a</sup>    | Hydrocarbons, 2-hydroxy-6-methylacetophenone   | Brophy et al. (1983)                      |
| <i>Rhytidoponera chalybaea</i> <sup>a,b</sup>  | Mellein, C <sub>13</sub> –C <sub>19</sub> alkanes, methyl-branched alkanes and alkenes, 3-methyl-2-cyclohexenone; 2,4-dihydroxyacetophenone              | Brophy et al. (1981), Sun and Toia (1993) |
| <i>Rhytidoponera metallica</i>                 | C <sub>13</sub> –C <sub>17</sub> alkanes, 2,5-dimethylchromone, isogeraniol  | Brophy et al. (1988)                      |
| Paraponerini                                   |  |   |
| <i>Paraponera clavata</i>                      | C <sub>16</sub> –C <sub>25</sub> alkanes and alkenes <sup>c</sup>  | Hermann et al. (1984)                     |
| Platythyreini                                  |  |   |
| <i>Platythyrea cribinodis</i>                  | Nothing volatile   | This work                                 |
| <i>Platythyrea punctata</i>                    | No Dufour gland  | This work                                 |
| Ponerini                                       |  |   |
| <i>Diacamma ceylonense</i>                     | C <sub>15</sub> –C <sub>27</sub> alkanes and alkenes   | This work                                 |
| <i>Diacamma indicum</i>                        | C <sub>13</sub> –C <sub>27</sub> alkanes and alkenes   | This work                                 |
| <i>Dinoponera australis</i>                    | C <sub>15</sub> –C <sub>21</sub> alkanes and alkenes, docosanol  | This work                                 |
| <i>Dinoponera grandis</i>                      | C <sub>15</sub> –C <sub>19</sub> alkanes and alkenes   | Hermann et al. (1984)                     |
| <i>Harpegnathos saltator</i>                   | C <sub>15</sub> –C <sub>25</sub> alkanes and alkenes, tetradecyl propionate  | do Nascimento et al. (1993)               |
| <i>Leptogenys diminuta</i>                     | C <sub>23</sub> –C <sub>27</sub> hydrocarbons and farnesenes   | Maile et al. (2000)                       |
| <i>Odontomachus bauri</i>                      | C <sub>15</sub> –C <sub>17</sub> alkanes and alkenes and three partially identified diterpenes   | This work                                 |
| <i>Odontoponera transversa</i>                 | C <sub>13</sub> –C <sub>19</sub> alkanes, methyl-branched alkanes, alkenes, a diterpene and geranylgeraniol  | This work                                 |
| <i>Pachycondyla apicalis</i> <sup>e</sup>      | C <sub>19</sub> H <sub>34</sub> , C <sub>20</sub> H <sub>38</sub> O <sup>d</sup> (revised formulae), also in <i>O. bauri</i> and <i>P. obscuricornis</i> | Cruz Lopez and Morgan, 1997               |
| <i>Pachycondyla castanea</i> <sup>e</sup>      | Nonanal, undecanal, isovaleric, nonanoic and phenylacetic acids, three amides and four amines  | Fales et al. (1984)                       |
| <i>Pachycondyla castaneicolor</i> <sup>5</sup> | The same three amides and four amines as found in <i>P. castanea</i>   | Fales et al. (1984)                       |
| <i>Pachycondyla indica</i>                     | C <sub>13</sub> –C <sub>23</sub> alkanes, methyl-branched alkanes and alkenes,   | This work                                 |

TABLE 9. CONTINUED

| Tribe, genus, species             | Chemicals   | Reference               |
|-----------------------------------|---|-------------------------|
| <i>Pachycondyla insularis</i>     | Gland atrophied   | Maschwitz et al. (1981) |
| <i>Pachycondyla obscuricornis</i> | 8 unidentified linear diterpenes  | This work               |
| <i>Pachycondyla striata</i>       | C <sub>15</sub> –C <sub>19</sub> alkanes, methyl-branched alkanes and alkenes, and five linear C <sub>25</sub> terpenes | This work               |
| <i>Pachycondyla tridentata</i>    | Gland atrophied   | Maschwitz et al. (1981) |
| Proceratiini                      |   |                         |
| <i>Proceratium itoi</i>           | No volatiles found  | This work               |

<sup>a</sup>Body extracted, but probably from the Dufour gland.

<sup>b</sup>Originally called *Rhytidoponera metallica* but corrected to *R. chalybaea* by Brophy et al. (1983).

<sup>c</sup>Information is unreliable and probably refers to cuticle.

<sup>d</sup>Unidentified terpenes.

<sup>e</sup>*Mesoponera* when published, the substances were found in total extracts of abdomen and thorax.

The tribe Ectatommini, according to Bolton's (1995) system, consists of six living genera: *Acanthoponera*, *Aulacopone*, *Ectatomma*, *Gnamptogenys*, *Heteroponera*, and *Rhytidoponera*. *Ectatomma* contains four known Neotropical species (*planidens*, *quadridens*, *ruidum*, and *tuberculatum*). The one we sampled is not one of these and must be a new species. There was nothing about its Dufour gland or mandibular glands that distinguished it chemically from examples from the large tribe of Ponerini, but *E. ruidum* belongs to the increasing number of ponerines that produce diterpenes. The three species of *Rhytidoponera* show some homogeneity by all producing in their Dufour glands acetophenone derivatives that are derived from a polyketide (Sun and Toia, 1993). The Asian species *Gnamptogenys menadensis* secretes a characteristic sticky substance from its pygidial gland, presumably serving as a repellent for potential predators. It also has some alarming and attractant properties (Gobin et al., 1998). Methyl 6-methylsalicylate, which is present, is a known alarm pheromone in the South American species *G. pleurodon* (Duffield and Blum, 1975), where it is found in the mandibular glands, but it has also been identified as a trail pheromone in the venom gland of the myrmicine ant *Tetramorium impurum* (Morgan and Ollett, 1987).

The tribe Paraponerini contains only the genus *Paraponera*. The Dufour glands of *Paraponera clavata* have been examined by Hermann et al. (1984) and the venom glands by Piek et al. (1991a,b). Their results, included in Tables 9 and 10, show similarities to some other ponerines.

The tribe Platythyreini contains only *Platythyrea* and *Probolomyrmex*. The Dufour glands of an unidentified species of *Platythyrea* from Kenya contain a series of alkenes and alkanes, with heptadecene providing over 50% of the total. *Platythyrea punctata* from Costa Rica is unusual in not possessing Dufour glands.

TABLE 10. SUMMARY OF KNOWN COMPOUNDS FOUND IN VENOM GLANDS OF PONERINE ANTS

| Tribe, genus, species          | Chemicals  | Reference  |
|--------------------------------|--|--|
| Amblyoponini                   |  |  |
| <i>Myrmium camillae</i>        | Nothing volatile   | This work  |
| Ectatommini                    |  |  |
| <i>Ectatomma tuberculatum</i>  | More than 15 polypeptides; complete sequence of ectatomin (molecular mass 7928 Da) has been solved   | Pluzhnikov et al. (1994), Zaitseva et al. (1995) |
| <i>Ectatomma</i> sp.           | Nothing volatile   | This work  |
| <i>Gnamptogenys menadensis</i> | Nothing volatile   | This work  |
| Paraponerini                   |  |  |
| <i>Paraponera clavata</i>      | Poneratoxin, at least 6 proteins <sup>a</sup> ; acidic proteins, some sequences determined   | Zaitseva et al. (1995)                           |
| Platythyreini                  |  |  |
| <i>Platythyrea cribinodis</i>  | Nothing volatile   | This work  |
| <i>Platythyrea punctata</i>    | Nothing volatile   | This work  |
| Ponerini                       |  |  |
| <i>Diacamma ceylonense</i>     | Undecanone   | This work  |
| <i>Diacamma indicum</i>        | Undecanone, methyl butanoate, 2-heptanone, heptanoic acid, 2,4,6-trimethylpyrazine, indole   | This work  |
| <i>Dinoponera australis</i>    | Nothing volatile, five proteins very similar to those of <i>Pachycondyla apicalis</i>  | Cruz López (1994)                                |
| <i>Dinoponera grandis</i>      | At least three proteins and glycoproteins by electrophoresis   | Leluk et al. (1989)                              |
| <i>Harpegnathos saltator</i>   | Nothing volatile   | do Nascimento et al. (1993)                      |
| <i>Leptogenys diminuta</i>     | (3 <i>R</i> ,4 <i>S</i> )-4-Methyl-3-heptanol  | Attygalle et al. (1988)                          |
| <i>Leptogenys peuqueti</i>     | 4-Methyl-7-tetradecanol and acetate, 4,10-dimethyl-7-tridecanol and acetate, 2,6-dimethyl-3-nonanyl acetate, 3,9-dimethyl-6-dodecanol, 6-methyl-3-nonanyl acetate, 4-methyl-7-dodecanol and acetate, 4-methyl-7-hexadecanol and acetate and two other related acetates and one alcohol | Janssen et al. (1997)                            |
| <i>Odontomachus bauri</i>      | Nothing volatile   | This work  |
| <i>Odontoponera transversa</i> | Many proteins and glycoproteins  | Leluk et al. (1989)                              |
| <i>Pachycondyla apicalis</i>   | 3-Benzyl-6-(2-methylpropyl)-2,5-piperazinedione, and a mixture of 5 proteins, very similar to those of <i>Dinoponera australis</i>   | Cruz López and Morgan (1997), Cruz López (1994)  |
| <i>Pachycondyla foetens</i>    | <i>N,N</i> -Dimethyluracil   | Janssen et al. (1995)                            |
| <i>Pachycondyla insularis</i>  | Two major and seven minor proteins   | Maschwitz et al. (1981)                          |

TABLE 10. CONTINUED

| Tribe, genus, species             | Chemicals   | Reference               |
|-----------------------------------|---|-------------------------|
| <i>Pachycondyla obscuricornis</i> | Benzaldehyde, 2,5-dimethylpyrazine, limonene, trimethylpyrazine, 2,5-dimethyl-3-ethylpyrazine, indole                                     | This work               |
| <i>Pachycondyla striata</i>       | Nonanal, decanal, and in some limonene  | This work               |
| <i>Pachycondyla tarsata</i>       | Dimethylaminoethanol, 2,6-dimethyl piperidine (two isomers), an amine, indole, <i>cyclo</i> -prolylleucine and sometimes other dipeptides | This work               |
| <i>Pachycondyla tridentata</i>    | Two major and seven minor proteins  | Maschwitz et al. (1981) |
| <i>Pachycondyla villosa</i>       | Rich in proteins <sup>a</sup>   | Blum and Hermann (1978) |
| Proceratiini                      |   |                         |
| <i>Proceratium itoi</i>           | Nothing volatile  | This work               |

<sup>a</sup>Unpublished results quoted by Blum and Hermann (1978).

The tribe Ponerini contains by far the most of the ponerine genera, 22 in all, of which we have analyzed 11 species. *Diacamma* is one of the ponerine genera that produce no true queens but have a reproductive system in which gamergates play a central role (Peeters and Crewe, 1986). Both *Diacamma ceylonense* and *Diacamma indicum* contained a complex mixture of linear hydrocarbons with just one methyl-branched alkane (Table 3). They were both unusual in having volatile compounds in their venom glands, and in both, 2-undecanone was the major substance (Table 10). This seemed particularly interesting since tandem recruitment has been demonstrated in a *Diacamma* species (Maschwitz et al., 1986). Of *Odonotomachus*, with over 100 species scattered throughout the tropics, *O. bauri* is the

TABLE 11. SUMMARY KNOWN COMPOUNDS FOUND IN PYGIDAL GLANDS OF PONERINE ANTS

| Tribe, genus, species          | Chemicals  | Reference                |
|--------------------------------|--|--------------------------|
| Amblyoponini                   | No reports   |                          |
| Ectatommini                    |  |                          |
| <i>Rhytidoponera metallica</i> | Isogeraniol, <i>m</i> -hydroxybenzaldehyde, heptadecene and heptadecadiene | Meinwald et al. (1981)   |
| <i>Gnamptogenys menadensis</i> | Methyl 6-methylsalicylate and unidentified $\delta$ -lactones              | This work                |
| Paraponerini                   | No reports   |                          |
| Platythyreini                  | No reports   |                          |
| Ponerini                       |  |                          |
| <i>Pachycondyla foetens</i>    | Actinidine   | Janssen et al. (1995)    |
| <i>Pachycondyla marginata</i>  | Citronellal, isopulegol  | Hölldobler et al. (1996) |
| Proceratiini                   |  |                          |
| <i>Proceratium itoi</i>        | Nothing volatile   | This work                |

only example of the genus yet examined. It had a mixture of hydrocarbons and diterpenes (Table 5). *Odontoponera transversa*, the only species in this genus, is found throughout the Indo-Malayan region. Its Dufour glands contain a complex mixture of hydrocarbons and three diterpenes (Table 6). Three species of *Pachycondyla*, all collected in India, were examined. They showed no great similarity in their Dufour gland chemistry. *P. indica* had only hydrocarbons, *P. striatus* had a mixture of hydrocarbons and diterpenes, and *P. obscuricornis* had only the complex mixture of diterpenes (Table 8). *P. obscuricornis* is recorded as another of the species that practices recruitment by tandem running (Hölldobler and Traniello, 1980). A curious form of defense has been recorded for *Pachycondyla*, whereby they produce a foam from the venom gland (Maschwitz et al., 1981). We looked particularly at *Pachycondyla tarsatus* venom glands because the venom of some of the ants was bitter-tasting, but not of others. All samples contained the cyclic dipeptide cycloleucylproline. This has been isolated from a number of sources, including the chrysalis of the silkworm moth *Bombyx mori* (Butenandt et al., 1951), together with cycloalanylleucine, cycloalanylproline, and cycloleucylvaline. No physiological role for them was stated. Cycloleucylproline was also isolated from the culture filtrate of a *Streptomyces* bacterium. The bitter tasting samples also contained cycloglycylproline and cycloalanylleucine. We could not relate the bitterness to any particular compound. All dipeptides with hydrophobic side chains are said to be bitter-tasting (Matoba and Hata, 1972). Cycloleucylproline has been shown to be a bitter compound formed by ageing in Japanese sake (Takahashi et al., 1974). From our data it is not possible to calculate whether the concentration of cycloleucylproline was above the taste threshold of 1.3 mM (Takahashi et al., 1974) in the venom. The bitter dipetide cycloleucylphenylalanine was earlier found in the venom of *P. apicalis* (Cruz López and Morgan, 1997).

The tribe Ponerini share a mixture of hydrocarbons and diterpenes in their Dufour glands. Chemically, the most interesting point is the frequent occurrence of diterpenes, rare in other subfamilies and best exemplified by *Pachycondyla obscuricornis*. The Ponerini are also noteworthy as the only tribe known so far to have volatile compounds in their venom glands, but this is not true of all species.

The tribe Proceratiini contains just *Discothyrea* and *Proceratium*, for which we have one example of the latter, but it was another species of ponerines that had no volatile compounds in the Dufour glands or venom glands.

Finally there are two monogeneric tribes, Thaumatomyrmecini containing the genus *Thaumatomyrmex*, and the tribe Typhlomyrmecini with the one genus *Typhlomyrmex*. Both of these are cryptic, and there are few data on them.

The contents of the Dufour glands of 26 species of ponerines have now been examined (Table 9), and we have been able to gather information on six of the eight tribes with 15 genera represented in this table. These examinations of the Dufour gland bring us no closer to an understanding of its possible functions. Some species contain compounds that could be considered as candidate pheromones, such as the

diterpenes of *Ectatomma ruidum*, *Pachycondyla apicalis*, and *P. obscuricornis*, or the acetophenone derivatives found in *Rhytidoponera* species. Others, such as the *Diacamma* and *Platythyrea* species, contain only alkanes and alkenes, which seem less likely potential pheromones because they are found so widely distributed among the Hymenoptera and insects generally, while others, like *Mystrium camillae* and *Proceratium itoi* have nothing volatile in the gland. One wonders what is different about *Platythyrea punctata* that it does not need this gland. Except for the epoxides of *Amblyopone reclinata*, the same or similar substances listed here have been found elsewhere among the Hymenoptera. All of the substances in Table 9 can be biosynthesized in the ant from acetate units, either through the fatty acid pathway for hydrocarbons, alcohols, and esters; through polyketides for the acetophenones of *Rhytidoponera* (Brophy et al., 1983; Sun and Toia, 1993); or through mevalonate or homomevalonate units for the terpenes. There is nothing to suggest from the structures that they are sequestered from plants or other invertebrates.

Since hymenopteran venom is usually an aqueous protein solution, it is more appropriate to ask why the venom glands should have any volatile substances at all. Some ant venom glands contain trail pheromones (Billen and Morgan, 1998), but these are usually just trace amounts. Many of the substances listed in Table 10, for example the pyrazines (Morgan, 1984), indole, and certainly the piperazinedione of *Pachycondyla apicalis* are made from aminoacids. This is as expected for a gland primarily designed for producing protein venoms. Analyses of pygidial gland are at too elementary a stage to draw any conclusions.

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