(Z, E)-α-farnesene, main constituent of the hypertrophied Dufour's gland of the major workers of *Pheidole pallidula* (Formicidae)

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Summary. In the European dimorphic ant, *Pheidole pallidula,* the Dufour's gland of the major workers is hypertrophied and secretes nearly pure (Z, E)-α-farnesene. This compound is also present in the Dufour's gland of the minor workers but constitutes only around 10% of the volatiles. Chemical polymorphism in *P. pallidula* is different from that already reported for species of *Pheidole* from the New World.

Key words. Pheidole pallidula; Formicidae; (Z, E)-α-farnesene; polymorphism; poison gland; Dufour's gland; pygidial gland.

The ant genus *Pheidole* is remarkable for the great morphological variability observed among workers in the same society. In the most evolved species, as in *Pheidole pallidula,* a clear-cut distinction is possible between two neuter subcastes: the minors and the majors, which differ not only in their overall size, but also in the relative proportions of their exoskeleton and internal organs[3,4].

Different roles are recognized for the minors and majors both within the nest and during foraging and in defense [5-12]. Not surprisingly, exocrine glands producing pheromones and defensive compounds are also specialized according to the subcaste, but rather unexpectedly, strong differences are observed from species to species. In *P. dentata,* the venom gland reservoir of the majors shows a hypertrophic development correlated with the production of skatole[13], a potent ant repellent also secreted in the defensive glands of other insects like the Trichoptera, *Pycnopsyche scabripennis*[14] and the Nevroptera, *Chrysopa oculata*[15]. The content of the venom glands of the majors of several other New World species also has a strong fecal odor, possibly due to skatole[8,12,16]. On the other hand, in the Neotropical ant, *P. biconstricta,* it is mainly the hypertrophied pygidial gland of majors which produces defensive compounds and alarm pheromone, minors having a proportionally smaller pygidial gland[17]. We report here on still another example of specialization of an abdominal exocrine gland, the Dufour's gland, in the majors of the European species, *P. pallidula.*

Societies were collected in the district of 'Tarn et Garonne' (in the southwest of France) and maintained in the laboratory. Dufour's and venom glands were dissected under water out of workers freshly killed by freezing, and were extracted in n-pentane. The pygidial glands were observed on 2-μm sections of abdomens fixed in glutaraldehyde-osmium and embedded in Spuur's resin.

All abdominal glands are larger in the majors but it is the Dufour's gland which shows the greatest development (fig.); on average it is more than ten times bigger than the gland of minors (table). This gland is proportionally even more developed in the majors than the head capsule, well known for its hypertrophic development. The increase of the poison gland reservoir in the majors is only moderate.

Its volume is less than twice that of the poison gland reservoir in the minors. It is in fact smaller than in the minors relatively to the overall size of the abdomen. The pygidial gland of the majors possesses more secretory cells, and its reservoir is somewhat larger. However, these glands are little developed in *P. pallidula* and no secretion could be collected for analysis.

The hypertrophy of the Dufour's gland in the majors is correlated with a chemical specialization. Capillary GC analysis (OVI, 150°) of the pentane extract of Dufour's glands shows the presence of a major compound which could be isolated after chromatography on silica gel (eluent: hexane/acetone 99:1). The ultraviolet absorption maximum at 233 nm (hexane) is indicative of two conjugated double bonds. The ^{1}H NMR spectrum (250 mHz, $CDCl_3$, TMS) is more informative. It shows four singlets of 3H each at 1.59, 1.63, 1.68 and 1.81 ppm, attributable to four methyl groups on double bond, as well as six vinylic protons. This suggests that the major compound may be an α-farnesene. Comparison of its ^{1}H NMR spectrum with that reported for (Z, E)-α-farnesene[18,19] indicates that it is identical to the latter. (Z, E)-α-Farnesene was also detected in the Dufour's gland of minors. In this subcaste, however, this compound represents only about 12% of the compounds observed in the GC, whereas in the majors it constitutes about 94%. From GC analyses, we estimated that the majors produce about 80 times more (Z, E)-α-farnesene than the minors. No striking differences between majors and minors could be observed in the chromatograms of their venom gland extracts. Skatole was not detected in the secretions. This does not mean that these glands have the same function in both subcastes. Experiments, which will be detailed elsewhere, have demonstrated that the venom gland of the minors secrete the trail pheromone (undetected in the chromatograms). Trail pheromone is not produced, or only in smaller amounts, in the poison gland of the majors.

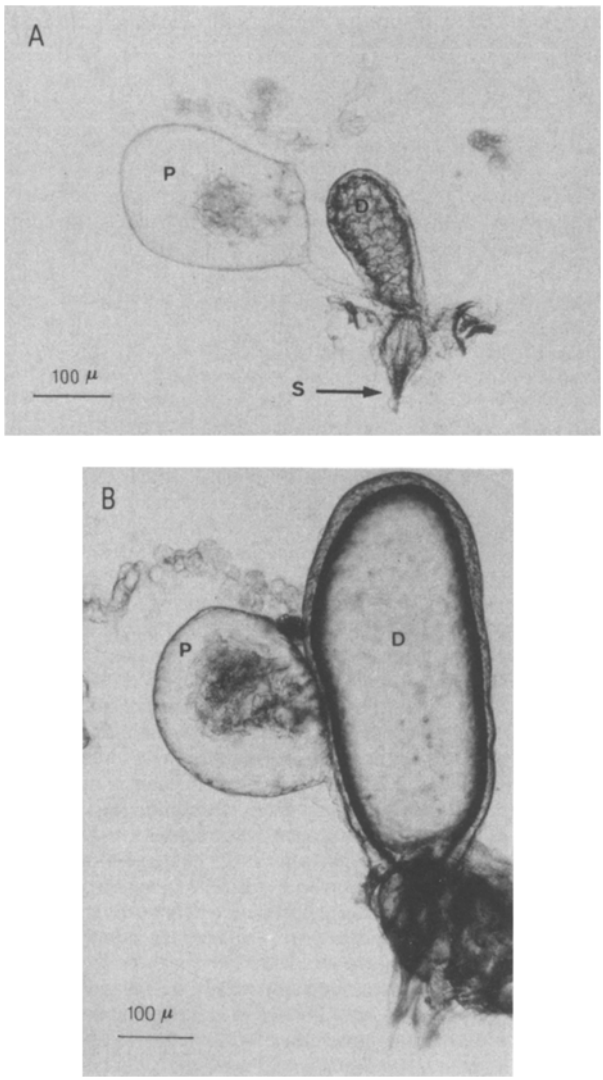
Farnesenes are not uncommon in the Dufour's gland secretion of ants, but they rarely constitute by themselves the bulk of the secretion. α-Farnesene constitutes 90% of the volatiles in the Dufour's gland of *Aphaenogaster longiceps*[20] and a farnesene (unspecified) is the major constituent of the Dufour's gland in the slave-raider ant *Polyergus rufescens*[21]. Homofarnesene, bishomofarnesene and (Z, E)-α-farnesene represent more than 80% of the glandular content in *Myrmica sabuleti, M. scabrinodis* and *M. schencki*[22]. (Z, E)-α-farnesene is also present in small quantities in the Dufour's gland of several *Myrmica* species and farnesene (not further specified) in *Formica* and *Componotus*[21,23]. For all these species, no behavioral function was attributed to those farnesenes. (Z, E)- and (E, E)-α-farnesene are constituents of the trail pheromone in *Solenopsis invicta*[19], but not in *P. pallidula.*

Majors of several *Pheidole* species play an important role in the defense of the society, and the specialization of their exocrine glands is usually correlated with this defensive function[8,10,12,17]. More research is needed, however, to interpret the biological meaning of the hypertrophy and the biochemical specialization of Dufour's gland in majors of *P. pallidula.* Interestingly, significant amounts of (Z, E)-α-farnesene have been detected in the defense gland of the Heteroptera, *Oxycarenus hyalinipennis*[24],

Volumes (in mm^3) of various organs in minors and majors of *Pheidole pallidula**

	Minor	Major	Ratio major/minor
Head capsule	$7.33\ 10^{-2} \pm 0.75\ 10^{-2}$(20)	$55.6\ 10^{-2} \pm 10.\ 10^{-2}$(20)	7.6
Dufour's gland	$1.14\ 10^{-3} \pm 0.30\ 10^{-3}$(14)	$12.80\ 10^{-3} \pm 6.38\ 10^{-3}$(36)	11.2
Poison gland reservoir	$2.45\ 10^{-3} \pm 0.67\ 10^{-3}$(14)	$4.61\ 10^{-3} \pm 1.66\ 10^{-3}$(47)	1.9

*Volumes were calculated with the formula valid for an ellipsoid. Sizes of the samples are given in the parentheses.



Poison gland reservoir (P) and Dufour's gland (D) associated with the sting (S) in *A* minor and *B* major of *Pheidole pallidula*.

and (E)-β-farnesene is an alarm pheromone in some aphids[25]. A function for (Z, E)-α-farnesene in the alarm-defense system of *P. pallidula* cannot be ruled out and is currently under investigation.

Our results are strikingly different from those obtained in the New World species in which some diversity has already been reported. Clearly, biochemical polymorphism and chemical defense has evolved several times in the genus *Pheidole*.

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1 Aspirante au Fonds National de la Recherche Scientifique.
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3 Wilson, E.O., Q. Rev. Biol. *28* (1953) 136.
4 Seynave, D., Mémoire de Licence, Université libre de Bruxelles 1983.
5 Creighton, W.S., and Creighton, M.P., Psyche *66* (1959) 1.
6 Creighton, W.S., Psyche *73* (1966) 1.
7 Calabi, P., Traniello, J., and Werner, M., Psyche *90* (1984) 395.
8 Hölldobler, B., and Möglich, M., Insectes soc. *27* (1980) 237.
9 Itzkowitz, M., and Haley, M., Insectes soc. *30* (1983) 317.
10 Wilson, E.O., Behav. Ecol. Sociobiol. *1* (1976) 63.
11 Droual, R., Behav. Ecol. Sociobiol. *12* (1983) 203.
12 Wilson, E.O., and Hölldobler, B., Insectes soc. *32* (1985) 3.
13 Law, J.H., Wilson, E.O., and Mc Closkey, J.A., Science *149* (1965) 544.
14 Duffield, R.M., Blum, M.S., Wallace, J.B., Lloyd, M.A., and Regnier, F.E., J. chem. Ecol. *3* (1977) 649.
15 Blum, M.S., and Wallace, J.B., Insect Biochem. *3* (1973) 353.
16 Wheeler, W.M., Ants: their Structure, Development and Behavior. Columbia University Press, New York 1926.
17 Kugler, C., Annls ent. Soc. Am. *72* (1979) 532.
18 Morgan, E.D., and Thompson, L.D., J. chem. Soc. P.I. (1985) 399.
19 Vander Meer, R.K., Williams, F.D., and Lofgren, C.S., Tetrahedron Lett. (1981) 1651.
20 Cavill, G.W.K., Williams, P.J., and Whitfield, F.B., Tetrahedron Lett. (1967) 2201.
21 Bergström, G., and Löfquist, J., J. Insect Physiol. *14* (1968) 995.
22 Cammaerts, M.C., Evershed, R.P., and Morgan, E.D., J. Insect Physiol. *27* (1981) 59.
23 Bergström, G., and Löfquist, J., Ent. scand. *3* (1972) 225.
24 Knight, D.W., Rossiter, M., and Staddon, B.W., J. chem. Ecol. *10* (1984) 641.
25 Bowers, W.S., Lowell, R.N., Webb, R.E., and Dutky, S.R., Science *177* (1972) 1121.

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