Comp. Biochem. Physiol. Vol. 100B, No. 4, pp. 667–680, 1991
Printed in Great Britain

0305-0491/91 $3.00 + 0.00

CUTICULAR HYDROCARBONS, SOCIAL ORGANIZATION AND OVARIAN DEVELOPMENT IN A POLISTINE WASP: *POLISTES DOMINULUS* CHRIST

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(*Received* 19 *June* 1991)

Abstract—1. The cuticular hydrocarbons of the wasp, *Polistes dominulus*, are linear branched, saturated alkanes, mainly monomethylalkanes.

2. The foundress can be distinguished from her offspring by differences in the relative proportions of some alkanes and monomethylalkanes, which were the same in all the foundresses studied here. The ovarian state is linked to the cuticular spectrum since these constituents were present in similar proportions in a foundress and in a descendant with comparably developed ovaries.

3. In some, but not all cases, it was possible to discriminate between descendants originating from different foundresses on the basis of other hydrocarbons belonging to all the chemical families present.

4. No correlations were observed between the descendants' behavioural profiles and the cuticular hydrocarbon spectra.

INTRODUCTION

Numerous data are available on the cuticular hydrocarbon spectra of termites and ants, which have been found to differ between the various categories (castes and sub-castes) of individuals in a colony as well as between colonies belonging to the same species. In wasps, however, this topic has been much less thoroughly documented so far.

Several authors have suggested that cuticular hydrocarbons may play a role in inter-individual recognition (see, for example, Blomquist and Dillwith, 1985). This has been shown to be the case in a few species: species recognition is now known to be based on cuticular hydrocarbons in termites [*Reticulitermes virginicus*, Howard *et al.*, 1982; *Reticulitermes* (*lucifugus*) *grassei* and *R.* (*lucifugus*) *banyulensis*, Bagnères *et al.*, 1991]; these substances are also involved in colony recognition and the exclusion of individuals from other colonies of the same species which have a different "colony odor" (Fielde, 1904), in several ant species (*Camponotus vagus*, Bonavita-Cougourdan *et al.*, 1987, 1988, 1989; *C. floridanus*, Morel *et al.*, 1988; *Cataglyphis cursor*, Nowbahari *et al.*, 1990).

The cuticular hydrocarbons, through their composition and/or their proportions, characterize the species, sometimes the population (*Camponotus vagus*, Bonavita-Cougourdan and Clément, 1986; *Cataglyphis cursor*, Lenoir *et al.*, 1988; Nowbahari *et al.*, 1990) and the colony to which these insects belong; in addition, within a single colony, their proportions can be specific to the caste (termites: Howard *et al.*, 1982; Bagnères *et al.*, 1991; ants: Mintzer *et al.*, 1987; Bonavita-Cougourdan *et al.*, 1990), the stage of development (termites: Howard *et al.*, 1982; Bagnères *et al.*, 1988; ants: Bonavita-Cougourdan *et al.*, 1988, 1990; Bagnères and Morgan, 1991; wasps: Espelie and Hermann, 1990), and the sex of its members (ants: Bonavita-Cougourdan *et al.*, 1990; wasps: Espelie *et al.*, 1990). In the ant, *Camponotus vagus*, moreover, the cuticular spectrum which is specific to each of the two castes (females and workers) varies depending on the physiological state of the individual in question. In the queen, these variations are linked to the egg-laying cycle. In the workers, they are probably related to the individuals' age and ovarian state, and differentiate between the two main behavioural sub-castes: brood-tenders and foragers. A relationship therefore exists in the worker class between the task distribution (polyethism) and the cuticular hydrocarbons. In all these cases, the particular cuticular hydrocarbons carried by all the members of a given colony are the same, but their proportions vary from one category of individual to another as well as being liable to fluctuate within each category (Bonavita-Cougourdan *et al.*, 1988).

These differences between the cuticular spectra which characterize various types of individual belonging to the colony may well be basic to the recognition processes at work within the colony and will consequently affect the way in which the colony functions and is regulated.

It seemed interesting to investigate whether the proportions of the various cuticular hydrocarbons carried by polistine wasps may serve to differentiate between the various categories of individuals belonging to a colony, as is known to occur among termites and ants.

Polistes wasps provide an interesting biological model for this type of study. They differ from most

other social insects in the following ways: their colonies are sparsely-populated and founded annually, there is very little polymorphism among the females, and the dominance–subordination scale or social hierarchy among the members of a colony is expressed in specific behaviours (Pardi, 1942, 1946, 1948).

In *Polistes dominulus* Christ [formerly *P. gallicus* (L.): Pardi, 1942, 1946, 1948], *P. fuscatus* and *P. canadensis* (West-Eberhard, 1969) nests are usually initiated by a single female, with which a few other mated females subsequently associate. Dominance–subordination relationships are then set up among the group of mated females, so that the colony becomes functionally monogynous. In the first place, the dominant α female is the most prolific: it inhibits the endocrine activity of the subordinate auxiliary females (Röseler *et al.*, 1984); the latter's ovaries degenerate and the number of eggs they lay gradually decreases (*P. gallicus*, Pardi, 1946, 1948; Gervet, 1962; Turillazzi and Pardi, 1977; *P. metricus*, Dropkin and Gamboa, 1981; *P. fuscatus*, Noonan, 1981). In the second place, the eggs laid by low-ranking females are destroyed by the dominant individual (Heldmann, 1936; Pardi, 1948; Gervet, 1964).

When the foundress and its associates (if any) disappear (or are removed for experimental purposes), one of the descendants accedes to the α rank. The descendants' hierarchical rank depends upon their order of emergence (*P. gallicus*, Pardi, 1948; *P. dominulus*, Théraulaz *et al.*, 1989; *P. exclamans*, Strassmann and Meyer, 1983; *P. instabilis*, Hugues and Strassmann, 1988).

Task distribution (polyethism) is known to take place within *Polistes* wasp colonies. Several different behavioural forms have been distinguished among the members of a recently founded colony (Théraulaz *et al.*, 1990) and those of a mature monogynous colony (Théraulaz *et al.*, 1991) by rating individuals on a large number of behavioural items, taking the duration and frequency of the behaviour into account. An individual's behavioural profile was found to depend to a large extent on its rank of emergence, and hence on its social status.

The aim of the present study was therefore to analyse the cuticular hydrocarbons carried by *Polistes dominulus* Christ wasps, and to investigate whether the proportions of the various components might vary in such a way as to differentiate: (i) colonies from each other; (ii) the queen (or foundress) from the other members of the colony; and (iii) some individuals from others performing different functions within the colony.

MATERIALS AND METHODS

Biological material

With a view to elucidating the above three points, we analysed the cuticular hydrocarbons of wasps from six colonies (a–f) and those of four foundresses (from colonies a, b, e and f); we also analysed the cuticular hydrocarbons of descendants (from colonies b, c and d), the exact age of which was known, and on which behavioural profiles had been drawn up during a prior observation period, using the method described by Théraulaz *et al.* (1991), amounting to a total of 52 wasps.

Colonies of two types were used for these studies:

(a) three young monogynous colonies, a, e and f (aged less than 1 month from the date of colony foundation), each produced by a single foundress. These colonies consisted of the foundress and the first four female descendants, the date of emergence of which was known: this made it possible to deduce their behavioural profiles (Théraulaz *et al.*, 1990);

(b) three mature colonies, b, c and d (aged 3 months from the onset of colony foundation), each of which was again produced by a single foundress: these colonies provided the individuals on which behavioural profiles were drawn up by observation. The foundress of one of these colonies (b) was sacrificed and its cuticular hydrocarbons analysed.

In all the colonies under investigation, the members were therefore closely related: the descendants were all sisters or half-sisters, and they were all daughters of the foundress.

The six colonies developed at the laboratory from foundresses collected while wintering in the South of France near Marseille, at two sites 4 km apart. Colonies a, b, c, e and f all originated from the one site and d from the other. The foundresses were first kept at the laboratory in a room with a constant temperature of 10°C and then placed in cages (16 × 19 × 24 cm) where they were provided with prey, blotting paper and sugar and the mean temperature was 27°C.

Behavioural profiles were drawn up during a 4-hr observation period and the wasps were immediately sacrificed by freezing. The four main behavioural profiles previously identified in mature colonies (Théraulaz *et al.*, 1991) were as follows:

—profile 1: characterizes the queen, which initiates more dominance-related activities and has the monopoly on egg-laying; it is also the individual with the highest overall level of activity within the nest;

—profile 2: characterizes individuals integrated into the colony which have a high level of overall activity on the nest surface and are often dominant; some of them are particularly oriented towards foraging activities;

—profile 3: characterizes individuals spending a high proportion of time off the nest or on the back of the nest, these are often inactive and rarely dominant;

—profile 4: characterizes individuals not integrated into the colony, which spend most of their time idling outside the nest.

Behavioural profiles 2, 3 and 4 all occurred in colonies b, c and d (20 individuals in all showed profile 2, 20 profile 3 and 12 profile 4). Profile 1 occurred in the foundress of colony b.

The mean age of all the wasps showing each of the four behavioural profiles was calculated. Comparisons were made as regards the state of ovarian development and the age-distribution of the individuals showing each type of profile, using the Mann–Whitney two-tailed non-parametric *U*-test.

The ovaries of each wasp studied were dissected out. The ovarian activity of each one was evaluated in terms of the mean length (*m*, expressed in mm) of the five largest oocytes among all those in the ovarioles; these large oocytes were mostly in the terminal position (cf. Röseler *et al.*, 1980).

In young colonies a, e and f, the foundress was numbered 1; the descendants were then numbered in the order in which they emerged (2–5). In the mature colonies, the foundress of colony b was numbered 1 and the b, c and d descendants were numbered at random: in colony b from 2 to 20, in colony c from 1 to 11 and in colony d from 1 to 22.

Chemical analyses

(1) The cuticular compounds were extracted from the head of each wasp by immersing them in 200 μl of pentane for 10 min. It was decided to analyse the hydrocarbons from the heads since that is where most of the wasps' partners

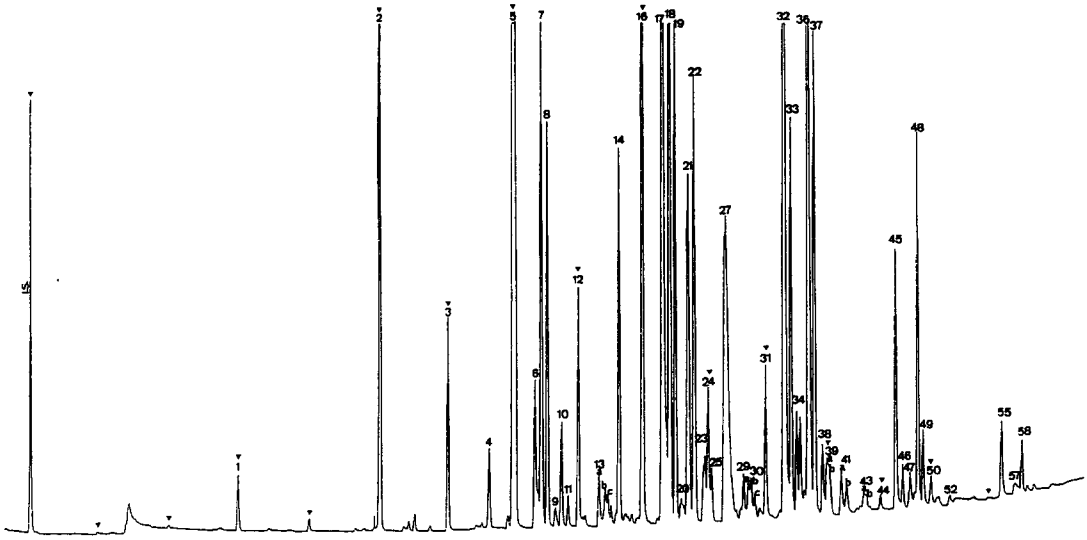

Fig. 1. Gas chromatogram of hydrocarbons from head cuticle of a descendant of *Polistes dominulus*. Analyses were realized on a fused silica capillary column with temperature program from 160 to 320°C at 3°C/min and vector gas was helium; the injection mode was splitless (15 sec).

carry out antennal exploration. To calibrate the different extracts an internal standard (nC_{20}, 100 ng) was added and then extracts were evaporated under nitrogen and adjusted at 50 μl. Two microliters of this solution was analysed by gas chromatography (G.C.) on a Delsi 330 GC, with a flame ionization detector (F.I.D.), equipped with a Chrompack capillary column CPSil5 WCOT (fused silica, 25 m × 0.25 mm). The injection mode was splitless (15 sec). The temperature programme was from 160 to 320°C at 3°C/min. The carrier gas was helium. Data were collected on a Enica 21 integrator. A standard alkane mixture from C_{20} to C_{36} was coinjected, and the equivalent chain length (ECL) calculated.

(2) For the mass-spectrometry analyses, live wasps from a colony originating from the same site as the experimental colonies were kept at the laboratory until ready for dissection. The insects were killed by freezing. Pieces of insect cuticle from various parts of the body were prepared and then chromatographed using the solvent-less technique as described by Bagnères and Morgan (1990). Gas chromatography–mass spectrometry was performed on a Hewlett-Packard 5890 GC coupled to a 5970B Mass selective detector (quadrupole M.S. using 70 eV electron impact ionization). The mass spectra were scanned from m/z 35 to m/z 500. The scan time was about 1.5 sec. Gas-chromatography was carried out on an immobilized polydimethyl siloxane phase (equivalent to OV 1) in a fused silica capillary column (12 m × 0.2 mm, 0.33 μm film thickness, SAC Chromatography), linked to the mass spectrometer by a 10 m length of 0.2 mm i.d. deactivated fused silica capillary (SAC Chrom.). Helium was used as carrier gas. The sample was heated for 5 min before being crushed in the injector at 280°C. The oven was programmed from 200°C (5 min isothermal) to 320°C at 3°C/min. The split vent was closed before crushing the sample and reopened 0.5 min later.

To complete the determination of the cuticular substances, a second series of analyses was carried out on a GC–MS Carlo Erba (Fisons) of the type QMD 1000 controlled by a LAB-Base data processing system. Here liquid pentane extracts from *P. dominulus* (heads of descendants from colony b) were injected on-column. The capillary column used was an RSL 150 (equivalent to OV 101, 30 m × 0.25 mm × 0.32 μm). The carrier gas was helium. After an initial electron impact analysis (EI at 70 eV, source temperature 160°C, interface temperature 330°C), a chemical ionization analysis was carried out (CI+, 75 eV, source temperature 200°C, interface temperature 330°C). The spectra were scanned with m/z ranging from 40 to 600. The extract was injected into the column at 60°C; after 1 min, the temperature was raised to 160°C at a rate of 25°C/min, and the analysis proper was carried out, increasing the temperature at a rate of 3°C/min up to 320°C, where it was maintained isothermally for a few minutes.

Identifications were based upon the mass spectra and compared with published results (Nelson and Carlson, 1986; Lange *et al.*, 1989; Page *et al.*, 1990; Grunshaw *et al.*, 1990). Determination of the 2-methyl branched alkanes was clear-cut and the results were compared with McDaniel's data (1990). Our results compared well with those by Nelson *et al.* (1988) on the retention time of various families of methylbranched hydrocarbons.

Data processing

For the chromatograms on each of the wasps studied, surfaces corresponding to the 56 main cuticular constituents determined with GC–MS given by the integrator coupled to the gas chromatograph were corrected using a flame ionization detector response coefficient as a function of the number of carbon atoms (Bagnères, 1989). The relative proportions of these substances, apart from the cholesterol (peak 29, Fig. 1), were calculated with Lotus 123 software. The results were analysed using simple (average and standard deviation) and multivariate procedures: Factorial Analysis of Correspondence (Biomeco Software) and Principal Components Analysis (Addad Software).

Several Factorial Correspondence and Principal Components analyses were carried out on 55 variables (the relative proportions of cuticular hydrocarbons) which were automatically normalized, from individuals of various origins: from the three young colonies (15 wasps), from the three mature colonies (53 wasps), and from the six colonies combined (68 wasps); the two types of analysis were also carried out on each of the mature colonies separately (colony b, 20 wasps; c, 11 wasps; d, 22 wasps).

A further Factorial Analysis of Correspondences was carried out on a condensed table of data giving the total proportions of each substance recorded in all the individuals in each colony (six colonies), taking into account neither the four foundresses (a1, b1, e1 and f1) nor wasps d7 and c11. All the individuals were processed as supplementary elements. Any one colony was therefore represented by a single dot in the individuals' space: its centre of gravity.

Table 1. Cuticular hydrocarbons of *Polistes dominulus*

Peak	Hydrocarbon	Ecl	CN	Diagnostic MS/EI ions	MS/CI+ ions	Percentage
1	*n*-Tricosane	23.00	23	324	323	0.24 (0.10)
2	*n*-Pentacosane	25.00	25	352	351	1.93 (0.80)
3	*n*-Hexacosane	26.00	26	366	365	0.77 (0.36)
4	2-Methylhexacosane	26.61	27	42/43, 336/337, 365	379	0.64 (0.37)
5	*n*-Heptacosane	27.00	27	380	379	10.53 (2.33)
6	13-, 11-Methylheptacosane	27.32	28	196/197, 224/225, 379	393	2.03 (1.2)
				168/169, 252/253, 379	393	
7	7-Methylheptacosane	27.42	28	112/113, 308/309, 379	393	0.59 (0.74)
8	5-Methylheptacosane	27.51	28	84/85, 336/337, 379	393	1.02 (0.52)
9	3-Methylheptacosane	27.64	28	56/57, 364/365, 379	393	0.22 (0.07)
10	5,15-Dimethylheptacosane	27.72	29	84/85, 196/197, 239, 351, 393	407	0.49 (0.35)
11	*x,y*-Dimethylheptacosane	27.83	29		407	0.14 (0.09)
12	*n*-Octacosane	28.00	28	394	393	1.20 (0.25)
13a	14-, 13-Methyloctacosane	28.32	29	210/211, 224/225, 393	407	0.60 (0.22)
				196/197, 238/239, 393	407	
13b	6-Methyloctacosane	28.45	29	98/99, 336/337, 393	407	0.10 (0.08)
13c	5-Methyloctacosane	28.50	29	84/85, 350/351, 393	407	0.09 (0.06)
14	2-Methyloctacosane	28.64	29	42/43, 365/366, 393	407	3.26 (1.08)
16	*n*-Nonacosane	29.00	29	408	407	4.28 (1.62)
17	15-, 13-Methylnonacosane	29.31	30	224/225, 407	421	12.42 (4.17)
				196/197, 252/253, 407	421	
18	7-Methylnonacosane	29.40	30	112/113, 336/337, 407	421	3.75 (1.69)
19	5-Methylnonacosane	29.51	30	84/85, 364/365, 407	421	1.84 (0.73)
20	9,15-Dimethylnonacosane	29.60	31	140/141, 224/225, 239, 323, 421	435	0.88 (0.54)
21	13,17-Dimethylnonacosane	29.73	31	196/197, 266/267, 421	435	2.34 (0.6)
	(and 3-Methylnonacosane and		30	56/57, 392/393, 407	421	
	7,13-Dimethylnonacosane)		31	112/113, 210/211, 253, 351, 421	435	
22	5,15-Dimethylnonacosane	29.80	31	84/85, 224/225, 239, 379, 421	435	1.64 (0.87)
23	Unknown	29.90				0.51 (0.20)
24	*n*-Triacontane	30.00	30	422	421	0.61 (0.23)
25	*x,y*-Dimethylnonacosane	30.02	31		435	0.25 (0.13)
27	15-, 14-Methyltriacontane	30.30	31	224/225, 238/239, 421	435	6.13 (3.17)
				210/211, 280/281, 421	435	
28	*x,y*-Dimethyltriacontane	30.60	32		449	0.27 (0.21)
30a	2-Methyltriacontane	30.67	31	42/43, 392/393, 421	435	0.30 (0.30)
30b	Hentriacontene	30.70	31	434	433	0.19 (0.11)
31	*n*-Hentriacontane	31.00	31	436	435	1.45 (1.92)
32	15-, 13-Methylhentriacontane	31.31	32	224/225, 252/253, 435	449	12.70 (3.72)
				196/197, 280/281, 435	449	
33	7-Methylhentriacontane	31.43	32	112/113, 364/365, 435	449	2.73 (1.25)
34	5-Methylhentriacontane	31.52	32	84/85, 392/393, 435	449	0.76 (0.49)
35	13,17-Dimethylhentriacontane	31.57	33	196/197, 224/225, 267, 295, 449	463	4.17 (1.91)
36	7,15-Dimethylhentriacontane	31.64	33	112/113, 238/239, 252, 379, 449	463	4.69 (2.25)
37	5,15-Dimethylhentriacontane	31.71	33	84/85, 238/239, 253, 409, 449	463	2.41 (1.09)
38	7,11,15-Trimethylhentriacontane	31.81	34	112/113, 182/183, 253, 323, 393, 463	477	0.51 (0.28)
39a	*n*-Docotriacontane	32.00	32	450	449	0.57 (0.32)
39b	*x,y*-Dimethylhentriacontane	32.05	33		477	
41a	16-, 14-Methyldocotriacontane	32.31	33	238/239, 252/253, 449	463	0.49 (0.20)
				210/211, 280/281, 449	463	
41b	8-Methyldocotriacontane	32.40	33	126/127, 364/365, 449	463	0.22 (0.12)
42	*x,y*-Dimethyldocotriacontane	32.59	34		477	0.18 (0.14)
43a	2-Methyldocotriacontane	32.64	33	42/43, 420/421, 449	463	0.11 (0.24)
43b	Tritriacontene	32.69	33	462	461	0.07 (0.20)
44	*n*-Tritriacontane	33.00	33	464	463	0.43 (1.14)
45	17-, 15-, 13-Methyltritriacontane	33.31	34	252/253, 463	477	2.92 (1.75)
				224/225, 280/281, 463	477	
				196/197, 308/309, 463	477	
46	7-Methyltritriacontane	33.43	34	112/113, 392/393, 463	477	0.49 (0.62)
47	13,17-Dimethyltritriacontane	33.56	35	196/197, 238/239, 281, 323, 477	491	1.75 (1.45)
48	7,15-Dimethyltritriacontane	33.72	35	112/113, 238/239, 281, 407, 477	491	1.59 (0.99)
	(and 5,15-Dimethyltritriacontane)			84/85, 252/253, 267, 437, 477	491	
49	7,11,15-Trimethyltritriacontane	33.82	36	112/113, 182/183, 253, 281, 351, 421, 491	505	0.49 (0.33)
50	*n*-Tetracontane	34.00	34	478	477	0.17 (0.12)
52	16-, 14-Methyltetracontane	34.34	35	238/239, 280/281, 477	491	0.11 (0.10)
				210/211, 308/309, 477	491	
55	17-, 13-Methylpentatriacontane	35.27	36	252/253, 280/281, 491	505	0.84 (0.58)
				196/197, 336/337, 491	505	
57	13,17-Dimethylpentatriacontane	35.51	37	196/197, 280/281, 267, 351, 505	519	0.60 (0.53)
58	7,15-Dimethylpentatriacontane	35.73	37	112/113, 238/239, 309, 435, 505	519	0.26 (0.20)
	and 5,15-Dimethylpentatriacontane			84/85, 238/239, 281, 463, 505	519	

Values of contributions to the axes were taken to be significant when equal to or greater than:

$$\frac{1000 \times 2}{\text{the number of variables}}.$$

Factor Analysis of Correspondences (FAC) (Greenacre, 1984; Lebart *et al.*, 1984) or reciprocal averaging (Hill, 1973) is now widely used with species and sample data (Gauch, 1982). Here we have applied the method to processing chemical data. It is an extension of Principal Component Analysis (PCA) which is well suited to dealing with either categorical variables or count variables. Its main feature is that it takes into account the margins of the data table, that is the sum of the scores on hydrocarbons and individuals. In Principal Components Analysis, there is only one weighting by the inverse of the standard deviation. In FAC the underlying distance function is said to be double-weighted. The distance between individuals i and i' is given by the formula:

$$D^2_{ii'} = \Sigma_j \frac{1}{x_j}\left(\frac{x_{ij}}{x_i} - \frac{x_{i'j}}{x_{i'}}\right)^2,$$

where x_{ij} is the score of hydrocarbon j in individual i; $x_i = \Sigma_j$ $x\ ij$ = sum of scores of individual i; $x_j = \Sigma_i\ x\ ij$ = sum of scores of hydrocarbon j.

RESULTS

Chemical determination

The GC–MS analyses showed that the compounds (apart from traces of alkene: 0.26%) were saturated alkanes, which were linear and branched (mono-, di-, trimethylalkanes) (Table 1). The molecular size ranged mainly from 23 to 37 carbon atoms (Fig. 1).

The cuticular mixture contained a high proportion of monomethylalkanes (54.4%), and *n*-alkanes and dimethylalkanes were present in roughly equal amounts (22.2 and 21.7%, respectively); trimethylalkanes amounted to only 1% of the total compounds.

The trimethylalkanes identified, 7,11,15-trimeC_{31} (peak 38) and 7,11,15-trimeC_{33} (peak 49) had their branches separated by three methylene groups (Page *et al.*, 1990; Lockey, 1991). The dimethylalkanes 13,17-dimeC_{29}, C_{31}, C_{33} and C_{35} have been previously observed in other polistine wasps, *Polistes annularis* (Espelie and Hermann, 1990) and *P. metricus* (Espelie *et al.*, 1990). Other alkanes with widely spaced branched methyls such as 5,15- and 7,15-dimethylalkanes of C_{29}, C_{31} and C_{33} (peaks 22, 36, 37 and 48) were identified as major constituents. Here the 2-methylalkanes (4.3%) occurred on the even alkyl chains of C_{26}, C_{28}, C_{30}, C_{32}, unlike those described by McDaniel (1990) in the termite *Coptotermes formosanus*, which showed a high proportion (about 20%) of 2-methylalkanes on the odd chains. An even series of the kind observed in the present study is also to be found in *Polistes biglumis bimaculatus* (Lorenzi and Bagnères, in preparation), as well as in the American *Polistes*, in small quantities. The *Polistes* wasps therefore do not seem to use the odd pathway via isovaleric acid described by Blailock *et al.* (1976), but rather that via isobutyric acid.

Foundresses, descendants, colonies and cuticular hydrocarbons

The Factor Analyses show that the cuticular hydrocarbon spectra of the foundresses differed from those of their female descendants in the proportions of some components. On the other hand, differences were observed between the cuticular characteristics of the offspring of one foundress and those of the others: these differences were quite marked in some cases and not in others.

Factor Analysis of Correspondences and Principal Components Analysis yielded similar results. The number of variables which yielded significant information was generally greater, however, with the latter than the former type of analysis.

(1) In the analysis of correspondences performed on *the three young colonies* originating from the same area (Fig. 2), axis 1 (42.2% of the inertia) separated the offspring from the foundresses (a1, f1, e1): the latter contributed most to this axis. The substances

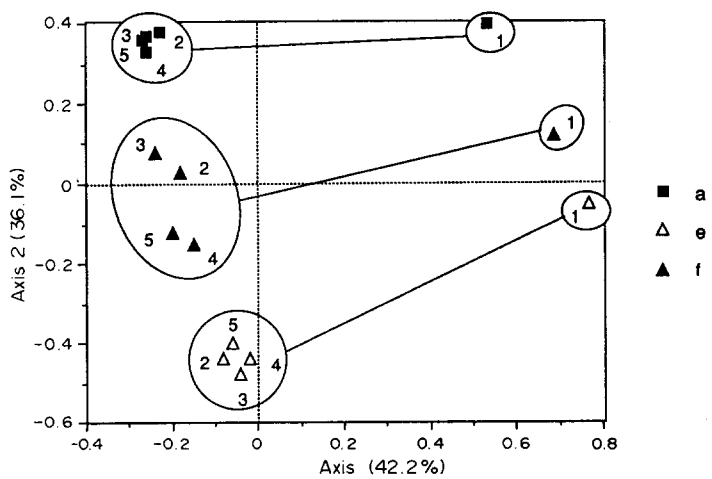

Fig. 2. Factorial correspondence analysis performed on three young colonies of *Polistes dominulus* (a, e and f), originating from the same area. Analysis was carried out on 55 variables (the relative proportions of cuticular hydrocarbons) automatically normalized, from 15 individuals, five belonging to each colony. 1, foundress; 2–5, descendants numbered according to their emergence rank. The envelope of each group was drawn arbitrarily for clarity.

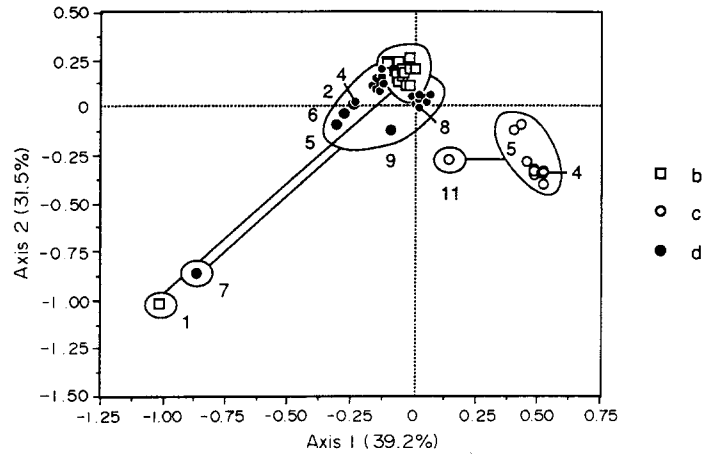

Fig. 3. Factorial correspondence analysis performed on individuals from three mature colonies of *Polistes dominulus* (b and c originated from the same area, d from another place). Analysis was carried out on 55 variables (the relative proportions of cuticular hydrocarbons) automatically normalized, from 53 individuals: 20 in colony b, 11 in colony c and 22 in colony d. b1, foundress of colony b with m (mean length of the five largest oocytes) = 1.80 mm; for other individuals b (descendants) $m < 0.81$ mm; c11, descendant of colony c with $m = 1.52$ mm; c4, $m = 1.35$ mm; c5, $m = 1.45$ mm; other descendants c, $m < 1.10$ mm; d7, descendant of colony d with $m = 1.70$ mm; d2, d4, d5, d6, d8, d9, 1.20 mm $< m <$ 1.63 mm. The envelope of each group was drawn arbitrarily for clarity.

which contributed most to the positive part of axis 1 (to which the foundresses projected) were: nC$_{31}$, 2-meC$_{32}$, nC$_{33}$, 17-meC$_{33}$ + 15-meC$_{33}$ + 13-meC$_{33}$, 7-meC$_{33}$ (peaks 31, 43a, 44, 45 and 46, respectively) and that which contributed to the negative part was 15-meC$_{29}$ + 13-meC$_{29}$ (peak 17). The substances which were most characteristic of the foundresses were therefore alkanes and monomethylalkanes.

Axis 2 (36.1% of the inertia) separated the offspring of three young colonies into three sets. The offspring in colonies a and e contributed the most to this axis, whereas those in colony f contributed the most to axis 3 (11.9% of the inertia). Axis 2 again separated foundress a (which contributed less to this axis than to axis 1) from foundresses e and f (which did not contribute to this axis at all). The constituents which contributed most to this axis in the case of its positive part (onto which the offspring and foundress of colony a projected) were: nC$_{27}$, 7-meC$_{27}$, 7-meC$_{29}$ and 15-meC$_{30}$ + 14-meC$_{30}$ (peaks 5, 7, 18 and 27), and in the case of its negative part (onto which the members of colony e projected): 15-meC$_{31}$ + 13-meC$_{31}$, 13,17dimeC$_{31}$ and 13,17-dimeC$_{33}$ (peaks 32, 35 and 47).

Axis 3 (11.9% of the inertia) separated colony f (including its foundress) from colonies a and e. The constituents which contributed the most to the positive part of the axis (onto which colony f projected) were: 15-meC$_{29}$ + 13-meC$_{29}$, 9,15-dimeC$_{29}$, 7,15-dimeC$_{31}$ and 5,15-dimeC$_{31}$ (peaks 17, 20, 36 and 37); those which contributed the most to the negative part of the axis, onto which colonies a and e projected, were: nC$_{25}$, nC$_{27}$, 15-meC$_{30}$ + 14-meC$_{30}$, 17-meC$_{33}$ + 15-meC$_{33}$ + 13-meC$_{33}$ and 13,17-dimeC$_{33}$ (peaks 2, 5, 27, 45 and 47).

From the Principal Components Analyses, two monomethylalkane mixtures in addition to those given above were found to characterize the foundresses: 16-meC$_{34}$ + 14-meC$_{34}$ (peak 52) and 17-meC$_{35}$ + 13-meC$_{35}$ (peak 55).

(2) The analyses of correspondences carried out on some individuals from *the three mature colonies* (two of which—b and c—originated from the same area as the young colonies, and the third—d—from another place) show (Fig. 3) that two individuals stood out sharply from the others analysed: b1, the foundress of colony b ($m = 1.80$ mm) and d7, the descendant in colony d with the most fully-developed ovaries ($m = 1.70$ mm).

These two wasps fell into the quadrant delimited by the negative parts of both axis 1 (39.2% of the inertia) and axis 2 (31.5% of the inertia). They both contributed considerably to these two axes, but more so to axis 2. The constituents most characteristic of these two individuals due to their quantitative preponderance were:

—nC$_{31}$, 7-meC$_{31}$, 7,15-dimeC$_{31}$, nC$_{33}$, 7-meC$_{33}$, 13,17-dimeC$_{33}$, 7,15-dimeC$_{33}$ + 5,15-dimeC$_{33}$ (peaks: 31, 33, 36, 44, 46, 47 and 48) which contributed strongly to the negative part of axis 1;

—17-meC$_{33}$ + 15-meC$_{33}$ + 13-meC$_{33}$ and 13,17-dimeC$_{35}$ (peaks 45 and 57), which contributed strongly to the negative part of axis 2. The hydrocarbons corresponding to peaks 31, 44 and 47 contributed to both axis 1 and axis 2, but more to axis 2 in the case of those corresponding to peaks 44 and 47.

Axis 1 also differentiates between two groups of individuals:

—those belonging to colony c, which contributed the most to this axis, apart from wasp c11 (which had the most fully developed ovaries of all the colony c individuals analysed, $m = 1.52$ mm), which contributed most to axis 4 (5.5% of the inertia);

—and those belonging to colonies b and d, which contributed little if at all to this axis but focused on axes 2 (31.5% of the inertia) and 3 (8.6%), 4 (5.5%), 5 (3.7%) and 6 (3.3%).

The constituents which contributed the most to the positive part of axis 1 (onto which the c individuals

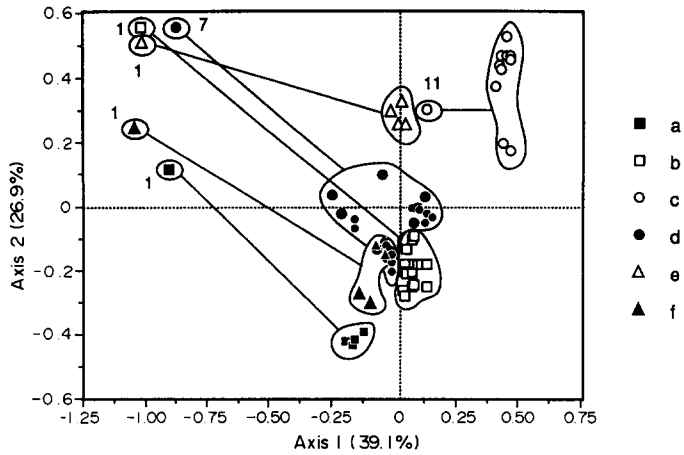

Fig. 4. Factorial correspondence analysis performed on all the studied individuals from the six colonies of *Polistes dominulus* (68 wasps). Legend as Figs 2 and 3.

projected) were: 13-meC_{27} + 11-meC_{27}, 15-meC_{29} + 13-meC_{29}, 15-meC_{31} + 13-meC_{31}, 13,17-dimeC_{31} and 13,17-dimeC_{33} (peaks 6, 17, 32, 35, 47).

Axis 2 (31.5% of the inertia) differentiated all the offspring of colonies b, d and c, which did not contribute to this axis, from wasps b1 and d7. These two wasps contributed the most to the negative part of this axis: the constituents which characterized them have been specified above.

Axis 3 (8.6% of the inertia) divided the foundress of colony b, which projected onto the positive part of the axis as well as contributing to it (less strongly, however, than to axes 1 and 2), from descendant d7, which projected onto the negative part and contributed nearly significantly to this axis. Constituents: $n$$C_{27}$, $n$$C_{29}$, 15-me$C_{29}$ + 13-meC_{29} and $n$$C_{31}$ (peaks 5, 16a, 17, 31) contributed to the positive part of the axis and can be said to have been characteristic of foundress b1, in view of their abundance; constituents: $n$$C_{25}$, 15-me$C_{31}$ + 13-meC_{31}, 5,15-dimeC_{31}, 17-meC_{33} + 15-meC_{33} + 13-meC_{33}, 13,17-dimeC_{33}, 7,15-dimeC_{33} + 5,15-dimeC_{33} (peaks 2, 32, 37, 45, 47, 48) contributed to the negative part of the axis and were characteristic of descendant d7. Alkane $n$$C_{31}$ (peak 31) and the mixture of monomethylalkanes of C_{33} (peak 45) were among the constituents characteristic of either the foundresses or the descendant with comparably developed ovaries: the alkane turned out to be most abundant in the foundress and the monomethylalkane mixture in the descendant. It should be remembered, however, that these hydrocarbons were present in greater quantities in these two individuals than in the b and d female descendants as a whole (cf. above and Fig. 2).

None of the six first axes (amounting to 92% of the inertia) separated colonies b and d completely from each other.

The Principal Components Analyses carried out on the mature colonies showed that two further constituents characterized the foundresses and the wasp d7: these were 16-meC_{34} + 14-meC_{34} (peak 52) and 17-meC_{35} + 13-meC_{35} (peak 55). These substances were also characteristic of the foundresses of the younger colonies.

The Factor Analysis of Correspondences and Principal Components Analysis both showed that some individuals belonging to colonies c and d differed from the other members of their colony. These were wasps c11, d9, d5, d4, d6 and d2. All of them had highly developed ovaries (mean length of the five largest oocytes: m = 1.52, 1.54, 1.27, 1.63, 1.20 and 1.47 mm, respectively). Two individuals in colony c which had relatively well-developed ovaries did not, however, stand out from their groups (c5, m = 1.4 mm; c4, m = 1.35 mm); in group d, the same occurred with wasp d8 (m = 1.29 mm). In all the other wasps in colonies b, c and d, m is less than 1.10 mm.

(3) In the analysis of correspondences carried out on *all the members of all six colonies* (Fig. 4), axis 1 (39.1% of the inertia) differentiated between three groups of individuals:

—the wasps in colony c, which contributed to the positive part of this axis (apart from wasp c11) as well as to axis 2;

—the foundresses and wasp d7 (which had highly developed ovaries), which contributed very strongly to the negative part of the axis;

—and a group of individuals from colonies a, b, d, e and f, which did not contribute to this axis.

Axis 2 (26.9% of the inertia) differentiated colonies e and c, which projected onto the positive part, from the offspring of colony a, which projected onto the negative part of the axis, as did a group of individuals from colonies b, d and f (Fig. 4). Descendants a, e and c contributed to this axis; but those from colony a contributed more to axis 4 (6% of the inertia), and those from e to axis 3 (8.1% of the inertia); while those from c made practically equal contributions to both axis 2 and axis 1.

Neither axis 3 nor axis 4 provided any means of discriminating between individuals belonging to colonies b, d and f.

The constituents contributing to axis 1 were: 13-meC_{27} + 11-meC_{27}, 15-meC_{29} + 13-meC_{29}, 15-meC_{30} + 14-meC_{30} and 13,17-dimeC_{31} (peaks 6, 17, 27 and 35) as far as the positive part was concerned (on to which wasps from c projected, and $n$$C_{31}$, $n$$C_{33}$, 17-me$C_{33}$ + 15m-C_{33} + 13-meC_{33}, 7-meC_{33} and 7,15-

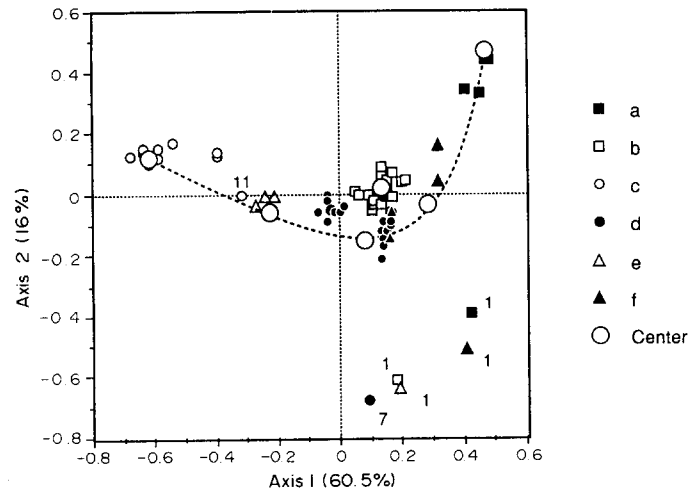

Fig. 5. Factorial correspondence analysis showing a Guttman's (1954) effect schematized by a dotted line. Analysis was carried out on a condensed table of data giving the total proportions of each substance recorded in all the studied individuals in each colony of *Polistes dominulus*, taking into account neither the four foundresses (a1, b1, e1 and f1) nor wasps d7 and c11. All the individuals were processed as supplementary elements.

dimeC_{33} + 5,15-dimeC_{33} (peaks 31, 44, 45, 46, 48) as far as the negative part was concerned (on to which the foundresses and wasps d7 projected). The constituents which contributed most strongly to the positive part of axis 2, onto which individuals e and c projected, were: nC_{31}, nC_{33}, 17-meC_{33} + 15-meC_{33} + 13-meC_{33}, 13,17-dimeC_{33} and 13,17-dimeC_{35} (peaks 31, 44, 45, 47 and 57); those which contributed most strongly to the negative part, onto which the a offspring projected were: 7-meC_{27}, 7-meC_{29}, 5,15-dimeC_{29} and 7,15-dimeC_{31} (peaks 7, 18, 22 and 36).

A similar analysis performed on a condensed table of data, in which wasps d7 and c11 were not taken into account, showed (Fig. 5) that a Guttman effect had occurred (Guttman, 1954): among the cuticular mixtures, there existed a gradient through which it was possible to move from colony c to colony a as follows: c, e, d, b, f, a.

Table 2. The most characteristic cuticular hydrocarbons present in the six colonies of *Polistes dominulus* studied, in terms of their amounts

Colony	Peak	Hydrocarbon
a	7	7-meC_{27}
	10	5,15-dimeC_{27}
b	13a	14-meC_{28} + 13-meC_{28}
	17	15-meC_{29} + 13-meC_{29}
	27	15-meC_{30} + 14-meC_{30}
c	6	13-meC_{27} + 11-meC_{27}
	32	15-meC_{31} + 13-meC_{31}
	35	13,17-dimeC_{31}
	47	13,17-dimeC_{33}
d	33	7-meC_{31}
	39ab	nC_{32} + x,y-dimeC_{31}
	46	7-meC_{33}
e	45	17-meC_{33} + 15-meC_{33} + 13-meC_{33}
	47	13,17-dimeC_{33}
	48	7,15-dimeC_{33} + 5,15-dimeC_{33}
	49	7,11,15-trimeC_{33}
f	20	9,15-dimeC_{29}
	21	13,17-dimeC_{29}
	25	x,y-dimeC_{29}
	36	7,15-dimeC_{31}

With the present method, the differences between the proportions of the various cuticular hydrocarbons from one member of the colony to another disappeared. The centre of gravity of each colony consequently made the strongest contribution to all or part of another axis. The centre of gravity of colony c contributed most to axis 1 (negative part), that of colony a and d to axis 2 (positive and negative parts, respectively), and that of colonies b and e to axis 3 (positive and negative parts, respectively); the centre of gravity of colony f contributed to the negative part of axis 4. Since monomethylalkanes and dimethylalkanes (Table 2) contributed strongly to either the whole or part of the axis onto which the centre of gravity of each colony projected, these constituents can be said to have been the most characteristic of each of the six colonies, quantitatively speaking.

Behavioural profiles and cuticular hydrocarbons

The Principal Components Analysis and Factor Analysis of Correspondences performed separately on each of the three groups of individuals from mature colonies did not bring to light the existence of any relationship between behavioural profiles and cuticular hydrocarbon spectra. The only clear-cut result to emerge here was the fact that in all cases, axis 1 separated the indiviudals with the most highly developed ovaries from the other members of the colony: foundress b1 (profile 1) and descendants d7 (profile 4) and c11 (profile 3) contributed the most to axis 1. The constituents which contributed most to this axis were practically the same between wasps b1 (the foundress, mean length of the five largest oocytes: m = 1.8 mm) and d7 (m = 1.7 mm), namely: nC_{31}, nC_{33}, 17-meC_{33} + 15-meC_{33} + 13-meC_{33}, 7-meC_{33} and 17-meC_{35} + 13-meC_{35} (peaks 31, 44, 45, 46 and 55). A different set of constituents, however, characterized wasp c11 (which had the most highly developed ovaries of all the c wasps, m = 1.52 mm),

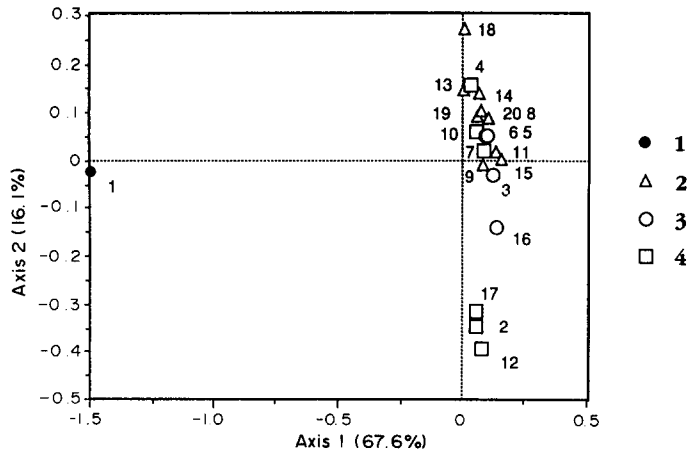

Fig. 6. Factorial correspondence analysis performed on b wasps (mature colony) of which behavioural profiles were drawn up during a 4-hr observation period before killing by freezing. Analysis was carried out on 55 variables (the relative proportions of cuticular hydrocarbons) automatically normalized, from 20 individuals: the foundress b1, behavioural profile 1; the descendants b8, 9, 11, 13, 14, 15, 18, 19 and 20, profile 2; the descendants b3, 5, 6 and 16, profile 3; the descendants b2, 4, 7, 10, 12, 17, profile 4.

namely: 7-meC_{29}, 5-meC_{31}, 7,15-dimeC_{31}, 5,15-dimeC_{31} and 7,11,15-trimeC_{31} (peaks 18, 34, 36, 37 and 38); one of these constituents (5-meC_{31}, peak 34) also contributed strongly to axis 1 in the case of wasp d7.

Within each colony, the group of individuals which contributed most to axes 2–6 did not form a separate group from the point of view of either the behavioural profiles or the age of its members. Colony b is a good example of this statement (Fig. 6).

Analysis of Correspondences on the members of colony b showed that axis 1 (67.6% of the inertia) set foundress b1 (profile 1), which contribute the most to this axis, apart from the other members of the colony. The constituents which contributed the most to this axis as regards the negative part onto which the foundress projected were: nC_{31} and nC_{33} (peaks 31 and 44), and to a lesser extent: nC_{29}, 17-meC_{33} + 15-meC_{33} + 13-meC_{33}, 7-meC_{33}, 17-meC_{35} + 13-meC_{35}, 13,17-dimeC_{35} (peaks 16a, 45, 46, 55 and 57); the constituents which contributed the most to the positive part of the axis were: 15-meC_{29} + 13-meC_{29} (peak 17).

Four wasps in colony b contributed strongly to axis 2 (16.1% of the inertia), which separated three of them (b2, b12 and b17) from the fourth (b18). Wasps b2 (age: 10 days), b12 (8 days) and b17 (9 days) had a type 4 behavioural profile, whereas wasps b4 (14 days), b7 (7 days) and b10 (6 days) had the same profile but contributed the most to axis 6 (b4 and b7) and 3 (b10) and little if at all to axis 2. Unlike wasp b18 (10 days) which had a type 2 behavioural profile, wasp b11 (9 days), b8 (9 days) and b15 (8 days), although they also had a type 2 behavioural profile, contributed only slightly if at all to axis 2; these wasps contributed, however, to axes 3 (b13), 4 (b15 and b20), 5 (b9, b11, b14 and b19), and 6 (b8) which, apart from axes 3 and 4 showed very low levels of inertia (less than 2.5% of the inertia). The wasps with profile 3, namely b3 (6 days), b5 (9 days), b6 (9 days) and b16 (6 days) contributed the most to axes 3, 5, 4 and 6, respectively.

Factor Analysis of the cuticular spectra did not show the existence of any important differences in the younger colonies between the most active members (numbers 2 and 3) and the individual which was the least well integrated into its colony (number 5) (Fig. 2).

Behavioural profiles, age and ovarian development

The ages of the wasps with each of the behavioural profiles characteristic of offspring were very variable in the three mature colonies, as reflected in the high values of the standard deviations. The wasps with profile 2 (nine of which belonged to colony b, four to colony c and seven to colony d) had a mean age of 19.1 days (SD = 10.5). Those with profile 3 (four of which belonged to colony b, six to colony c and 10 to colony d) had a mean age of 8.4 days (SD = 8.6). Those with profile 4 (six of which belonged to colony b, one to colony c and five to colony d) had a mean age of 9.9 days (SD = 7.7). The ages of the wasps with profile 2 differed significantly from those of the wasps with profiles 3 and 4. No significant difference was observed, however, between the ages of the wasps with profiles 3 and 4 (significance level <0.05, Mann–Whitney's two-tailed U-test). Wasps d7 and c11, the cuticular spectra of which differed from those of the other members of the respective colonies, were aged 3 and 32 days, respectively. Foundress b, which had a type 1 profile, was more than 250 days old, as were the foundresses of young colonies a, e and f.

As regards the possible existence of correlations between the behavioural profile and the extent of ovarian development, the mean length (m) of the five largest oocytes (which was taken as an index to ovarian development) of the offspring with behavioural profile 2 was 0.63 mm (SD = 0.34). In the offspring with profile 3, it was 0.74 mm (SD = 0.46), and in those with profile 4, it was 0.51 mm (SD = 0.50). Comparisons between these values representing three different behavioural profiles yielded no significant differences (significance level <0.05, Mann–Whitney's two-tailed U-test).

On the other hand, we compared the offspring of all the colonies from the point of view of their ovarian development (the standard deviation was calculated only when the number of individuals was much greater than 10). The mean length of the five most fully developed oocytes was 0.48, 0.36 and 0.38 mm in the offspring of young colonies a, e and f, 0.42 mm (SD = 0.21) in the offspring of colony b, 0.78 mm (SD = 0.45) in those of colony c, and 0.77 mm (SD = 0.50) in those of colony d. No significant differences emerged from comparison between the values of *m* recorded in all the descendants of the younger colonies combined (a, e and f) and those of each mature colony separately (b, c and d) (significance level <0.05, Mann–Whitney's two-tailed *U*-test). The maximum values recorded in colonies a, e, f and b were 0.54, 0.64, 0.45 and 0.81 mm, respectively; in the case of four wasps in colony c and eight wasps in colony d, the values of *m* were greater than the maximum value recorded in the offspring in colony b (colony b was the same age as colonies c and d). For the sake of comparison, it should be remembered that in the foundress queens, the value of *m* ranged between 1.75 and 1.90.

DISCUSSION

The cuticular hydrocarbons carried by *Polistes dominulus* are of the same kind as those previously described in other *Polistes* (Espelie and Hermann, 1990; Espelie *et al.*, 1990). The major components present tend to differ, however, from one species to another: in *Polistes metricus*, the major components are two *n*-alkanes (nC_{27} and nC_{29}) and two mixtures of central monomethylalkanes of C_{31} and C_{33} (11-meC_{31} + 13-meC_{31} + 15-meC_{31} and 11-meC_{33} + 13-meC_{33} + 15-meC_{33} + 17-meC_{33}); in *Polistes annularis*, terminally-branched methylalkanes of C_{27} and C_{29} (3-meC_{27} and 3-meC_{29}) and a centrally-branched methylalkane of C_{31} (13,17-dimeC_{31}) predominate; in *Polistes dominulus*, the major constituents are an *n*-alkane (nC_{27}), mixtures of centrally-branched monomethylalkanes of C_{29} and C_{31} (15-meC_{29} + 13-meC_{29} and 15-meC_{31} + 13-meC_{31}) and a dimethylalkane of C_{31} (7,15-dimeC_{31}).

The cuticular hydrocarbon spectra (i.e. 55 constituents or mixtures covering practically the whole spectral range) of *Polistes dominulus* wasps belonging to different colonies and different castes or exhibiting different behavioural patterns within a colony were compared by performing Factor Analysis of Correspondences and Principal Components Analysis on the experimental data.

(1) These analyses showed that the foundress-queens differed noticeably from their descendants (Figs 2–6) in the proportions of a few of their cuticular hydrocarbons, which were always the same ones from one colony to another. These individuals were characterized by an abundance of alkanes nC_{31} (peak 31) and nC_{33} (peak 44) and monomethylalkanes 17-meC_{33} + 15-meC_{33} + 13-meC_{33} (peak 45), 7-meC_{33} (peak 46), 16-meC_{34} + 14-meC_{34} (peak 52) and 17-meC_{35} + 13-meC_{35} (peak 55); and by the paucity of another mixture of monomethylalkanes, 15-meC_{29} + 13-meC_{29} (peak 17).

This particular type of cuticular spectrum was not actually restricted to the foundress queens alone: one of the descendants the ovarian development of which ($m = 1.70$ mm) was comparable in size to that of the foundresses (*m* from 1.75 to 1.90 mm) within a mature colony showed a cuticular hydrocarbon spectrum which was very similar to that of those foundresses (Figs 3–5); the constituents which differed between the other descendants of this and other colonies were the same as those which demarcated the foundress-queens from the descendants.

The descendants with medium-developed ovaries (*m* from 1.10 to 1.63 mm) differed slightly from the other descendants (at least in most cases), but even more so from the foundresses (Figs 3 and 4) in their cuticular mixtures.

A close relationship can therefore be said to have existed between the ovarian state and the cuticular spectrum of these wasps. Now the ovarian development is known to be under the control of the neuroendocrine system: in associated *Polistes* foundresses, a correlation exists between the relatively large size of the corpora allata, the relatively large quantities of juvenile hormone synthesized, the holding of α rank, and the reproductive activity (*Polistes gallicus*, Pardi, 1980; Röseler *et al.*, 1980; Turillazzi *et al.*, 1982; *Polistes metricus*, Ross and Gamboa, 1982); the production of ecdysteroids, which is correlated with the oocyte development, is also higher in α-ranking foundresses than in the other females (Strambi *et al.*, 1977). It is likely that the quantitative variations in the cuticular hydrocarbons carried by *Polistes dominulus* females (both foundresses and descendants) may be under hormonal control. It is also quite plausible that descendants acceding to the α rank, which always have highly developed ovaries, will also exhibit the hydrocarbon spectrum typical of foundress-queens.

Several authors have previously shown that in insects the production of cuticular hydrocarbons is under hormonal control (Jallon *et al.*, 1981; Adams *et al.*, 1984; Blomquist *et al.*, 1984; Trabalon *et al.*, 1990; Schal *et al.*, 1991). Trabalon *et al.* (1990) have shown in particular that in *Calliphora vomitoria* Diptera, previtellogenesis is concomitant with an increase in the proportions of some of the hydrocarbons composing the cuticular mixture (*n*-alkanes and monomethylalkanes), whereas those of other constituents (alkenes and dimethylalkanes) remain unchanged. In *Polistes dominulus* wasps, there also seems to exist a threshold in hormonal state (to which the size of the oocytes serves as an index) which coincides with the presence in specific proportions of some of the cuticular hydrocarbons.

Definite evidence that changes in hydrocarbon synthesis are under hormonal control can only be obtained by inducing changes in the hormonal titre of wasps with underdeveloped ovaries.

The cuticular odour characteristics of the foundress or the descendant which accedes to α rank upon the death or experimental removal of the former, thus becoming responsible for egg-laying, might constitute the chemical signal (or one of the signals) which is known to provide recognition of social status in the wasp *Polistes fuscatus* (Downing and Jeanne, 1985). We have observed, however, that

in a mature colony, a descendant can be both capable of egg-laying and at the same time have a cuticular odour which is very similar to that of the foundress-queen when the latter still occupied the nest. Comparative analysis between the ovarian development of the offspring of that colony and that of younger colonies suggested that the foundress in question was no longer able to inhibit the ovarian development of its own daughters. It would be interesting to investigate whether the existence of the α odour in several members of a single colony (as long as the foundress has kept its α odour but lost the ability to inhibit the ovarian development of its offspring) results in any increase in the number of dominance–subordination interactions, and possibly in the disorganization of the social hierarchy within the colony or the setting up of a new pattern of organization.

(2) As regards the colony odour in *Polistes dominulus* wasps, all the factor analyses showed that a sharper distinction exists between foundress-queens and their offspring than between one colony and another (i.e. between one family line and another).

These analyses, in which all the wasps participating in this study (members of six colonies) were taken into account, show that the cuticular hydrocarbons sometimes characterize in their proportions all the descendants of a single foundress. In some cases it is not possible, however, to distinguish clearly between the offspring of various foundresses on the basis of their cuticular hydrocarbons (Fig. 4). The workers from three of the six colonies investigated (a, c and e) were distinguishable from each other on the basis of their cuticular spectra, whereas the other three (b, d and f), given the inter-individual differences existing within each colony, had such similar cuticular compositions that none of the first six axes (which accounted on average for more than 85% of the total inertia) separated them from each other.

Within the whole population of wasps studied, the cuticular spectra of the descendants of each colony formed a continuum, as shown by the Guttman (1954) effect observed (Fig. 6).

The main constituents which characterized individual colonies differed from those which characterized the foundresses. These substances belonged to all the chemical families represented here, namely: one *n*-alkane (nC_{32}), monomethylalkanes of C_{27}, C_{28}, C_{29}, C_{30}, C_{31} and C_{33}, dimethylalkanes of C_{27}, C_{29}, C_{31} and C_{33} and one trimethylalkane of C_{33}.

The size of the variations in the descendants' cuticular spectra was not attributable here, where the wasps originated from a small geographical area, to the place of origin of the foundresses from which they descended: colonies b and d, which had very similar spectra, were started by foundresses collected at sites which were 4 km apart, whereas colonies a and c, which had very different spectra, descended from foundresses which had been collected at the same site.

In *Polistes dominulus* wasps, the female descendants of one foundress can have a highly particular odour, but, as we have seen, this was not always the case: some of the family lines did not comply with this rule, in that their cuticular hydrocarbon proportions were all very similar. If discrimination between nestmates and non-nestmates (here between related and unrelated or more distantly related individuals) is based on the cuticular hydrocarbon mixture, then there exist cases where the wasps cannot perform this discrimination efficiently. Now these hydrocarbons do seem to be involved in *Polistes* wasps' recognition processes: the nesting paper is known to be impregnated with the same hydrocarbons as those which are to be found on the cuticles of the workers, larvae and eggs (*P. annularis*, Espelie and Hermann, 1990) in proportions which are fairly similar to those of the adult cuticle; and these hydrocarbons account for the preference shown by 66% of the wasps for their own nest (*Polistes metricus*, Espelie *et al.*, 1990). In *Polistes metricus*, it is also possible that the mixtures of cuticular hydrocarbons from some colonies may be very similar.

Discrimination processes have been found to take place among nestmate (assumed to be close kin) and non-nestmate workers in several wasp species, such as *Polistes fuscatus* (Pfennig *et al.*, 1983) and *Ropalidia marginata* (Venkataraman *et al.*, 1988), but the statistical results obtained by these authors do not make it possible to ascertain whether discrimination actually always took place. Other signals in addition to those consisting of cuticular hydrocarbons may have made this discrimination possible.

(3) Lastly, no correlation was found to exist between the wasps' cuticular mixtures and their behavioural profiles, the latter being determined on the basis of the frequency and the duration of a large number of items in their behavioural repertoire (Fig. 6), at least, insofar as these could be detected with the type of factor analysis used here. It is worth noting, however, that wasps with the same behavioural profile differed as regards their age and their ovarian state. Those individuals which were the most active on the nest and which took part in foraging (behavioural profile 2) were nevertheless older than those which were less active (behavioural profile 4); in our sample, age was not the differentiating factor between behavioural profiles 3 and 4. Nor did the wasps' ovarian development seem to differ between behavioural profiles 2, 3 and 4. It should be remembered that wasps' ovarian state varies depending on their age: the female offspring's ovarian activity increases during the first 10 days of life, and varies considerably thereafter from one individual to another (Pratte *et al.*, 1982). In wasps which form small-sized annual colonies, the behaviour of the individual members shows considerable plasticity, which makes it possible to adapt quickly to changes in the surroundings (Théraulaz, 1991).

(4) It might be interesting to compare these data on polistine wasp colonies with the relevant data on ants.

From the data obtained here on *Polistes dominulus*, discrimination between nestmates and non-nestmates on the basis of the cuticular hydrocarbons did not always seem to be possible, when the foundresses of the colonies investigated were collected from sites in the same vicinity. In ants (*Camponotus vagus*, Bonavita-Cougourdan *et al.*, 1986; *Cataglyphis cursor*, Nowbahari *et al.*, 1990), when two colonies show aggressive behaviour towards each other, the underlying discrimination and recognition processes have been found to involve differences in the composition of the cuticular hydrocarbon mixtures from

one colony to another. Colonies which are closely related geographically—and possibly genetically—tend, however, to be much less aggressive towards each other, or even to be mutually tolerant, and correlatively, their cuticular hydrocarbon spectra tend to show strong similarities.

As occurs in ants (at least in *Pseudomyrmex ferruginea*, Mintzer *et al.*, 1987; *Camponotus vagus*, Bonavita-Cougourdan *et al.*, 1989), the foundress-queens in *Polistes dominulus* colonies have characteristic cuticular hydrocarbon spectra which generally differ from those of their offspring. The differences between the spectra of a foundress and her female descendants are greater, however, in ants than in wasps. These differences are liable to play a role in foundress recognition, possibly along with other cues, particularly those relating to the foundress' specific behavioural patterns. Contrary to what happens among ants, however, the polistine female descendants belonging to mature colonies can develop ovaries which are almost as large as those of the foundresses, as well as having similar cuticular hydrocarbon spectra, without necessarily showing a behavioural profile of the foundress type. This particularity of polistine wasps may be attributable to their very low degree of polymorphism and the great variability of the descendant wasps' ovarian development.

The last point worth mentioning here is that contrary to what has been observed in the ant *Camponotus vagus*, the cuticular hydrocarbons of *Polistes dominulus* do not seem to characterize the individuals in the colony which have the same behavioural profile at a given moment. In polistine wasps, the cuticular hydrocarbons do not therefore seem to be involved in behavioural sub-caste recognition (if any such process exists) whereas in ants recognition processes of this kind have been found to exist and have been correlated with differences in the cuticular mixture (Bonavita-Cougourdan *et al.*, 1986).

Acknowledgements—We thank Professor E. D. Morgan for his help and use of his equipment. This research was partially supported by a Singer-Polignac Foundation Grant.

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