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Cuticular lipids as semiochemicals in paper wasps and other social insects

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Strong evidence indicates that in *Polistes* wasps, as in other social insects, epicuticular lipids are involved in several aspects of recognition, such as nest-, nestmate- and fertility recognition. This evidence is based both on the study of differences in composition between groups of conspecific individuals (members of different colonies; age groups; dominants and subordinates; fertile and infertile individuals), and on bioassays. The first part of this review considers the general characteristics of *Polistes* epicuticular hydrocarbons and summarises what is currently known about the non-hydrocarbon cuticular lipids. The second part concerns the most relevant contributions of the work on *Polistes* towards an understanding of the role of epicuticular hydrocarbons as semiochemicals in social insects. Four aspects, highlighted in 1993 by Howard, as future directions for the study of epicuticular hydrocarbons will be considered: (i) the need for a complete determination of epicuticular hydrocarbon structures (in particular chirality); (ii) the role of individual hydrocarbons as semiochemicals, or of classes of hydrocarbons in the epicuticular mixtures; (iii) the intraspecific variability in epicuticular lipid composition; and, (iv) current knowledge about cuticular hydrocarbon perception by insects.

Introduction

Since the early 1990s, an increasing volume of research has been conducted on the epicuticular lipids of insects and has examined their role as semiochemicals. Until then epicuticular lipids had received little attention, and the work on insect semiochemistry had been mainly focused on more volatile compounds, generally secreted and released, by well-defined exocrine glands.

The interest in epicuticular lipids has been particularly strong among students of social insects. For social insects, the presence of "superficial odours" acting as recognition cues has been known since the work of Adele Marion Fielde (1904, 1905) on nestmate recognition in ants. A surge of interest in kin recognition occurred in the 1980s, about two decades after Hamilton's paper on kin selection was published (1964). The interest in social insect recognition systems, combined with the availability of bench instruments for the analysis of volatile and semi volatile compounds, and the straightforward identification of the epicuticular components, led to an increase in the number of papers published on social insects epicuticular lipids.

In *Polistes* wasps, nestmate recognition has been studied since the beginning of the 1980s (*see* Gamboa 1996, 2004). Several aspects of the origin of recognition cues (Gamboa *et al*. 1986a) and of the ontogeny of nestmate recognition (Gamboa *et al*. 1986b) had already been clarified when the first papers on *Polistes* epicuticular lipids were published (Espelie & Hermann 1990, Espelie *et al*. 1990, Bonavita-Cougourdan *et al*. 1991, Singer & Espelie 1992, Singer *et al*. 1992). This was probably one of the reasons why the first papers on *Polistes* epicuticular lipids focused on the role of these compounds as nestmate recognition pheromones, and often included bioassays aimed to investigate this behaviour.

The work on kin recognition in social wasps has been reviewed by Gamboa (Gamboa *et al*. 1986b, Gamboa 1996, 2004, Singer *et al*. 1998). Other reviews have considered the role of epicuticular hydrocarbons in social insects (Lorenzi *et al*. 1996, Singer 1998), and have also discussed the work done with *Polistes* wasps. Very recently, an important section of the review by Howard and Blomquist (2005) on insect cuticular hydrocarbons was dedicated to social insects, including, of course, paper wasps. Although there is, perhaps, little need for a further review on epicuticular lipids in *Polistes* wasps, this contribution deals with some aspects that have not be considered, or treated only briefly, before. The first part of this review will consider the general characteristics of *Polistes* epicuticular hydrocarbons and summarise what is currently known about the non-hydrocarbon cuticular lipids. In the second part, I attempt to highlight the most relevant contributions made by the work on *Polistes* epicuticular hydrocarbons to our general understanding of the role of these compounds as semiochemicals in social insects. To do so, I use as a guide the paragraph titled “Future Needs and Directions” written by Howard (1993) in his review on cuticular hydrocarbons and chemical communication.

Polistes epicuticular lipids: cuticular hydrocarbons and a few oxygenated compounds

Hydrocarbons

Of the 206 *Polistes* species, the composition of epicuticular lipids has been reported for the adult females of only four American and seven European species, including the three *Polistes* social parasites. Table 1 shows the principal characteristics of the epicuticular lipid composition of these species. A compound quantification based on a correction for the detection of *n*-alkanes with different chain length has been used in the studies of the American species (*see* references quoted in Table 1), of *P. dominulus* (Bonavita-Cougourdan *et al*. 1991), and of *P. biglumis bimaculatus* (Lorenzi *et al*. 1997), while no corrections were adopted in the other studies. Although analytical devices (gas chromatographs coupled to flame ionization detectors GC-FID or to mass spectrometers GC-MS) generally show a decreasing response to *n*-alkanes when the chain length increases, the adopted corrections do not consider a possible differential response to distinct classes of hydrocarbons (alkanes, alkenes, centrally and terminally methyl-branched alkanes), due to their different physico-chemical properties (Gibbs & Pomonis 1995). Despite disparities in the quantification methods, the data reported in Table 1 are suitable for showing both the common features and the differences between species.

The general features of the *Polistes* hydrocarbon profiles correspond to those described for many insect species where methyl branched alkanes predominate (Nelson 1978, Lockey 1988, Carlson *et al*. 1998). In all *Polistes* species examined (Table 1), the range of carbon atoms in the main chain is similar, and methyl branched alkanes, represented by numerous compounds (*see* Bonavita-Cougourdan *et al*. [1991] for an example of a very detailed list), constitute a high percentage of the total quantity of hydrocarbons. The high number of methylalkanes, and their high abundance with respect to *n*-alkanes and alkenes, is a unifying characteristic of analysed *Polistes* wasps. With respect to the other two polistine species for which the epicuticular hydrocarbons have been reported, *Polistes* are similar to *Polybia occidentalis*, with respect to the presence of methylalkanes (*see* Singer *et al*. 1998), but very different from *Parachartergus aztecus,* where only *n*-alkanes and *n*-alkenes were identified (Espelie & Hermann 1988). Among the five stenogastrine species analysed so far (Zanetti *et al*. 2001, Cervo *et al*. 2002, Beani *et al*. 2002,

Table 1. Major characteristics of epicuticular lipid composition in *Polistes* species.

P. annularis Espelie & Hermann 1990[c,d,e]	*P. exclamans* Singer *et al.* 1992[c,d]	*P. fuscatus* Espelie *et al.* 1994[c,e]	*P. metricus* Layton *et al.* 1994[c,e]	*P. dominulus* Bonavita *et al.* 1991[c]	*P. biglumis* Lorenzi *et al.* 1997[c]	*P. nimphus* Turillazzi *et al.* 1998[g]	*P. atrimandibularis* Fanelli 2001[f]	*P. gallicus* Fanelli 2001[g]	*P. sulcifer* M. F. Sledge *et al.* unpubl. data[f]	*P. semenowi* Lorenzi *et al.* 2004[g]
n-Alkanes relative abundance and chain length										
16.4	18.9	71.1	46.7	19.33	31.2–32.87	C25–C30	43.5	43.8	27.84	C23–C34
C23–C29	C23–C31	C21–C31	C23–C33	C25–C33	C23–C33		C23–C32	C23–C32	C23–C31	
n-Alkenes relative abundance and chain length										
0	0.7	0.18	7.9	0		C31	29.0	0.1	0.28	
	C27–C29	C29	C23–C35				C27–C29	C27		
Methyl–alkenes relative abundance and main chain length										
0	0	4	0	0	0	0	0	0	0	0
		C29–C31								
Centrally methyl branched alkanes relative abundance and main chain length										
14.22	49.4	10.5	18.68	43.07	36.88	C27–C33	10.4	31.1	41.44	C25–C35
C27–C33	C29–C33	C29–C33	C27–C35	C25–C35	C25–C35		C25–C31	C25–C33	C25–C33	
Terminally methyl branched alkanes[a,b] relative abundance and main chain length										
34.15	18.1	0	0.42	7.77–12.26	6.48–8.52	C26–C30	3.9	7.8	13.21	C26– C34
C25–C31	C27–C29		C27	C26–C29	C24–C32		C26–C27	C27–C29	C26–C30	
Dimethyl alkanes[b] relative abundance and main chain length										
35.19	7.1	14.19	22.44	19.1–24.7	21.34–24.38	C29–C35	13.2	17.1	16.40	C27–C35
C29–C33	C29–C33	C31–C33	C31–C35	C27–C35	C25–C35		C27–C31	C27–C33	C27–C33	
Trimethyl alkanes relative abundance and main chain length										
0	0	0	1	1.0	0.2	C31–C33	0	0	0	0
			C35	C31–C33	C33					
Range of carbon atoms in the main chain										
C23–C33	C25–C33	C21–C33	C23–C35	C25–C35	C23–C35	C25–C35	C23–C32	C23–C33	C23–C33	C23–C35
Oxygenated compound										
			Heneicosanal Tricosanal	Methyl oleate[i,l] Octadecenal[i] Octadecanal[i] 1–Octacosanol[m] 1–Triacontanol[m] Long chain Fatty acids[l] Long chain fatty Ammides[n]					Methyl oleate[n] Octadecenal[n] Octadecanal[n] Long chain fatty Ammides[n]	

[a] 3- and 2-methyl alkanes, according to Lockey 1988. [b] Terminal monomethyl branched alkanes and some dimethyl branched alkanes are often in coelution and therefore is not possible to specify the relative abundance of each. Here the relative abundance is given as an interval. [c] Percentages were calculated by considering a response coefficient for the different classes of hydrocarbons. [d] Data refer to workers only. [e] Percentages did not sum to 100 in the original paper and were here reported to 100. [f] Data refer to parasite before invading the host colonies [g] Data refer to foundresses only. [h] Percentages not reported. [i] Dani *et al.* 1996, 2003. [l] Dani *et al.* 2003. [m] Sledge *et al.* 2001. [n] F. R. Dani *et al.* unpubl. data.

Turillazzi *et al.* 2004), branched alkanes have only been found in *Liostenogaster vechtii* (Turillazzi *et al* 2004) and *Parischnogaster mellii* (Beani *et al.* 2002), the latter of which presents several mono- and dimethyl-alkanes. Of the five Vespinae analysed, *Vespa crabro* presents a low number of methyl-branched alkanes (mainly terminal monomethylalkanes), while *n*-alkanes and *n*-alkenes constitute a high percentage of the total amount of hydrocarbons (Butts *et al.* 1991, Butts & Espelie 1995, Dani *et al.* 2004b). In the other four species (Brown *et al.* 1991, Butts *et al.* 1991), the number of methylalkanes and their relative amount is higher, but the pattern appears less complicated than in *Polistes*.

In *Polistes*, monomethyl alkanes occur both as iso- and anteiso- alkanes (respectively position 2 and 3, both indicated by Lockey [1988] as terminally branched methylalkanes) and centrally branched methylalkanes. For some alkanes with an odd number of carbons in the main chain (which, in most insects, predominate), the whole series of positional isomers bearing the methyl substituent in odd positions can be found. The methylalkanes with an even-numbered backbone bear the methyl group on both even and odd positions, but the terminal branched isomer is generally an isoalkane.

Most of the reported dimethylalkanes are compounds with odd-numbered backbones, bearing both methyl groups centrally, in odd positions and always separated by at least 3 methylenes. Compounds represented by 7,y- and 5,y-dimethyl alkanes (where y often corresponds to position 15), have also been reported. Only three terminal dimethylalkanes, 3,y-C_{29}, 3,y-C_{31} and 3,y-C_{33} have been reported (Lorenzi *et al.* 1997, 2004a, Sledge *et al.* 2001). Since these compounds are seen in low amounts, and elute (on apolar polydimethylsiloxane GC columns, commonly used for cuticular hydrocarbon analyses) after the linear alkane having an additional carbon with respect to those in the main chain, their identification may be difficult and may have been unidentified in several cases. Some even-numbered main-chain dimethylalkanes have been reported, but in only a few cases have the branching positions been determined (Lorenzi *et al.* 1997, 2004a). Only a few trimethylalkanes have been listed, and they are always among the compounds with the higher number of carbons in their backbone (*see* Table 1). The trimethyl alkanes whose structure has been reported (Bonavita-Cougourdan *et al.* 1991, Lorenzi *et al.* 1997, 2004a) all have an odd number of carbons in their main chain, odd branching positions, and three methylenes between the branching points.

It should be noted that, papers reporting on the same species might differ in a few of the reported compounds, in particular dimethylalkanes (Bonavita-Cougourdan *et al.* 1991, Sledge *et al.* 2001, 2004, Dani *et al.* 2004b, Lorenzi *et al.* 2004a). This may, partially, be due to the fact that several positional isomers of dimethylalkanes, especially the centrally branched, may coelute in the same chromatographic peak rendering mass spectral interpretation difficult. In addition, authors may report only the compounds for which the spectra are more intense. However, differences between samples used in the studies may be biologically relevant, and this possibility should be investigated (*see* also the section “Cuticular hydrocarbon mixture variability”).

With the exception of *P. metricus* and the social parasite *P. atrimandibularis,* the concentration of alkenes is low in all the species (*see* Table 1). *Polistes atrimandibularis* females analysed before invasion of the host colonies, appear quite unique among the species studied due to the high percentage of alkenes. The concentration of alkenes decreases drastically once the parasite becomes integrated within its host colony (Bagnères *et al.* 1996, Fanelli 2001). Only in *P. atrimandibularis* have the positions of the alkene double bonds been determined (Bagnères *et al.* 1996), and these are all centrally located along the chain, as is the case for many insects (Blomquist *et al.* 1980, Blomquist & Dillwith 1985, Lockey 1988). Although (*Z*)-isomers are the alkenes commonly found in epicuticular lipids (Blomquist *et al.* 1980), stereochemistry has not been investigated in *P. atrimandibularis.*

Two methyl-branched alkenes have been found in *P. fuscatus* (Espelie *et al.* 1994), but details about their identification were not given. Methyl-branched alkenes have rarely been reported among insect epicuticular hydrocarbons (Brown *et al.* 1990, Howard 1993, Fukaya *et al.* 1996), and there is little information about both their mass spectra and their chromatographic behaviour.

Hydrocarbon biosynthesis has never been studied in *Polistes* or in other social wasps, although such studies would be of great interest especially for social parasites (*see* Lorenzi, 2006). These data may help us understand the mechanisms by which social parasites acquire the same epicuticular profile as their host colonies. Biosynthesis of long chain hydrocarbons in insects has been reviewed by several authors (Nelson 1978, Blomquist & Dillwith 1985, Blomquist *et al.* 1987, Lockey 1988, Nelson 1993), most recently by Blomquist and Howard (2003), Morgan (2004), and Howard and Blomquist (2005).

In *Polistes*, the Dufour gland has been shown to contain the same hydrocarbons as those found on the cuticle (Dani 1995, 1996a), although in slightly different percentages. It is unknown, however, if the gland itself secretes these compounds, or, as is the case for the honeybee, these compounds are sequestered into the gland from the hemolymph (Katzav-Gozansky *et al.* 2000).

Only in a few species have epicuticular lipids been examined in males. In *P. annularis* (Espelie & Hermann 1990), two methylalkanes were reported for males but not for females, although most compounds were common to both sexes. Importantly, a clear difference in the concentration of several compounds was observed between these two groups. Qualitative differences were not observed between *P. metricus* males and females, but males had a much higher concentration of alkenes than females (Layton *et al.* 1994). A similar trend was observed for two subspecies of *P. major* (quoted in Layton *et al.* 1994).

Most of the compounds reported for the cuticle of adults have also been found in the extracts of *P. annularis* eggs and larvae, and no peculiar compounds were reported for these two immature stages (Espelie & Hermann 1990). Larval extracts were analysed in *P. fuscatus* by Panek *et al.* (2001), and they contained the same hydrocarbons as adult females. Egg and larval hydrocarbons were also analysed in *P. biglumis* (Lorenzi 1992), and although a list of compounds was not reported, the profiles of these two samples were similar to those of adult females.

The nest is also covered with hydrocarbons found on the animal cuticle (Espelie & Hermann 1990, Espelie *et al.* 1990, Singer *et al.* 1992, Lorenzi 1992, Fanelli 2001, Lorenzi *et al.* 2004a), and a few extra hydrocarbons are often found on the nests (Espelie *et al.* 1990, Espelie & Hermann 1990, Singer *et al.* 1992, Lorenzi *et al.* 2004a). These additional compounds are generally in low amounts, and may be undetectable or unidentifiable on the insects. The role of the nest hydrocarbons in nest and nestmate recognition has been reviewed by Singer *et al.* (1998), while Gamboa (2004) has recently resumed research on their origin.

Oxygenated compounds

In addition to hydrocarbons, some oxygenated compounds have been reported in a few species (Table 1). These are always minor constituents relative to the hydrocarbons. Oxygenated compounds appear to be variable within a species, as they are reported in some papers but not in others. No correlation between the presence of these compounds and any particular factor (such as population or physiological state) has been reported. It should be noted that in most work, epicuticular extracts are prepared using apolar solvents, such as pentane or hexane, which are more effective in removing hydrocarbons than more polar lipids. Specific experiments intended to analyse oxygenated compounds found on *Polistes* epicuticular lipids have never been reported. Although hydrocarbons are the most common compounds in *Polistes*, and evidence indicates that they are involved in nestmate recognition (Dani *et al.* 2001), a close inspection of oxygenated compounds on *Polistes* wasps and their possible role as recognition cues should be conducted. It is worth remembering that, although hydrocarbons have been demonstrated to act as nestmate recognition cues in four ant species (Lahav *et al.* 1999, Wagner *et al.* 2000, Akino *et al.* 2004, Ozaki *et al.* 2005), oxygenated compounds appear to be more important than hydrocarbons for nestmate recognition in *Apis mellifera* (Breed 1998b, 1998a). In *P. dominulus*, young wasps exposed to a nest applied with a fatty acid ester (methyl pentacosanoate), preferred to associate with nestmates that had acquired this compound (Pickett *et al*. 2000). This experiment, although conducted with a compound never found on the cuticle or the nest, demonstrated that compounds other than hydrocarbons may be important as recognition cues.

Fatty aldehydes are the most commonly reported oxygenated compounds. Octadecenal and octadecanal have been found in *P. dominulus* (Dani *et al*. 1996a), and in *P. sulcifer* females collected from host colonies at the end of the colonial cycle (Dani 1995). Two aldehydes with an odd number of carbon atoms have been reported in *P. metricus* (Layton *et al*. 1994), and these are quite unusual, since insect fatty aldehydes, and fatty alcohols, generally have an even number of carbons in their backbone (Buckner 1993). Two primary aliphatic alcohols have been found in *P. dominulus* (Sledge *et al*. 2001).

Some fatty acids in the Van der Vecht glands (mainly palmitic (hexadecanoic), linoleic ((*Z,Z*)-9,12-octadecadienoic), and oleic ((*Z*)-9-octadecenoic) acids) have been reported in *P. annularis* (Espelie & Hermann 1990), *P. dominulus,* and *P. sulcifer* (Dani *et al*. 1995). These compounds were also found on the nest pedicel (Espelie & Hermann 1990, Dani *et al*. 1995). Since the unsaturated acids act as ant repellents (Dani *et al*. 1996b), the fatty acids were considered a gland secretion, which, once spread on the hairs of the cuticle around the gland's opening, was applied on the nest pedicel to repel predatory ants (Dani *et al*. 1996b). The presence of these compounds on the cuticle has been reconsidered, however, and data indicate that they may derive from contamination by internal tissues (Dani *et al*. 2003).

Fatty acid amides, and in particular oleamide, have been found on the cuticle and in the Dufour gland of *P. sulcifer* (Dani 1995), and occasionally in the body of *P. dominulus* (F. R. Dani unpubl. data). In an analysis of endogenous ligands of odorant binding proteins (OBP) in *P. dominulus*, oleamide and other fatty acid amides have been identified in the dichloromethane extracts of OBPs purified from female wings (Calvello *et al*. 2003). Fatty acid amides are very unusual components for epicuticular lipids, and since they are reported as contaminants from plastic materials (Ende & Spiteller 1982), their occasional presence in epicuticular extracts has been considered unnatural. Recently, however, a series of primary fatty acid amides has been reported as major components for a psocopteran (Howard & Lord 2003), and erucyl amide has been reported for *Anopheles gambiae* hexane body extracts (Caputo *et al*. 2005).

Chirality in branched hydrocarbons

The need to investigate chirality in methyl branched alkanes was highlighted by Howard (1993). In fact, methyl-branched alkanes, which are the major constituents of epicuticular lipids in many insects, contain chiral centres, but very little is known about their stereochemistry. Chirality has an important role in insect chemical communication (Mori 1984), but it has received little attention in the study of cuticular hydrocarbons. Determination of absolute configurations through comparison with synthetic standards analysed on chiral columns is commonly used in the study of chiral pheromones. Moreover, gas chromatography coupled to an electroantennography apparatus (gas chromatography electro antennography detection, GC-EAD), using chiral columns, is used to investigate insect perception of different enantiomers.

One of the reasons for the scarce information on chirality in epicuticular hydrocarbons is because no one has been able to separate enantiomers and diasteroisomers of long chain methylalkanes on chiral gas chromatographic columns. The separation of three forms of C_{17} dimethylalkane using a modified cyclodextrin chromatographic phase was recently reported ((7*R*,11*R*), (7*S*,11*S*)- and *meso*-7,11-dimethylheptadecane; Chow *et al*. 2004). The *meso*-form of this methylalkane, combined with (7*S*)-methylheptadecane, are sex pheromones of two geometrid species, and their activity has been demonstrated using synthetic compounds in both GC-EAD and field trapping (Duff *et al*. 2001). In another geometrid species, GC-EAD and field experiments were used to evaluate the effect of the different forms, synthetically prepared, of another dimethyl-branched C_{17} (3,13-dimethylheptadecane) sex pheromone (King *et al*. 1995). A longer monomethylalkane (13-methylhentriacontane), reported as a kairomone in two *Microplitis* braconid species, was studied for the effect of its enantiomers on arrestment behaviour, finding no differences between its two forms (Lewis *et al*. 1988). Three forms of 13,23-dimethylpentatriacontane were assayed as female sex pheromones in the fly *Glossina pallidipes*, finding that only the *meso*-isomer was active (McDowell *et al*. 1985, Naoshima & Mukaidani 1987).

With regard to social insects, the synthesis of the enantiomers of some monomethylalkanes (Marukawa *et al*. 2001) and of the four stereoisomers of 3,12-dimethylheptacosane (Masuda & Mori 2002), all compounds present on the cuticle of *Diacamma* ant queens, have been reported. It is thought that these compounds are involved in queen recognition (Masuda & Mori 2002), but bioassays using different forms have not been reported. Monomethylalkanes have been tested for their role in nestmate recognition in *P. dominulus* (Dani *et al*. 2001), but these were always racemic mixtures.

Role of individual hydrocarbon components in the semiochemical message

Insect epicuticular hydrocarbons are generally very complex mixtures, but the importance of individual components has rarely been investigated. As noticed by Howard (1993), this may be due both to the effort required to obtain the chemicals (through collection from GC or by synthesis) and to the difficulty of designing suitable bioassays. In social insects, where the epicuticular hydrocarbons have been studied in many species, only a few experiments have attempted to assess the importance of single components. In these organisms, although the relative abundance of the components has been found to differ in different conspecific groups (as is the case when comparing, for example, different colonies of a single population, individuals belonging to different castes, or fertile and infertile females), the observed variation is generally seen in several compounds and only rarely are they restricted to a limited number. Therefore, it is difficult both to hypothesize about the importance of single components and to set up appropriate bioassays.

Cases where differences between two groups are very evident, and restricted to one or a few compounds, have been reported for some ponerine ants (Monnin *et al*. 1998, Liebig *et al*. 2000, Dietemann *et al*. 2003, D'Ettorre *et al*. 2004), where gamergates are characterised by a high concentration of a compound absent or in very low amounts in infertile workers. On the contrary, when differences between fertile and infertile individuals have been studied in *Polistes* wasps, which like ponerine ants are characterised by a non-rigid differentiation between fertile and infertile females, the differences between the two groups were always found to be more complex (Espelie *et al*. 1994, Layton *et al*. 1994, Sledge *et al*. 2001, Beani *et al*. 2002, 2004, Dapporto *et al*. 2004c).

In a work investigating the compounds most relevant for nestmate and kin recognition in *P. fuscatus*, Espelie *et al*. (1994) found that the three most characteristic compounds were methylalkanes. The relative abundance of these compounds was important in classifying individuals to their own colony and to sister colonies (that is, to colonies founded by females which had been nestmates in the previous season), but did not differ in concentration between queens and workers. The authors suggested that the relative abundance of these compounds is a heritable trait and thus are suitable candidates as recognition pheromones. These authors also suggested that because of their structure, methylalkanes may be more suitable as pheromones than *n*-alkanes.

In a study on the same species (Gamboa *et al*. 1996), a sample of wasps was tested in recognition bioassays in which resident foundresses interacted with niece workers resident in another nest and then animals were analysed for the composition of their epicuticular hydrocarbons. By applying a Stepwise Discriminant Analysis using the absolute difference between the relative concentration of each compound in the incoming wasp and the average concentration of the same compound in the receiving colony as predictors, and the observed reaction by foundresses (rejection, acceptance) as the grouping variable, the authors explained all the observed behaviour. Indeed, the authors found that two of the most important hydrocarbons in the analyses were the same methylalkanes found to be important in the previous study; others were linear alkanes and compounds previously concluded to be of no importance.

Bioassays to test the role of some specific hydrocarbons in the context of nestmate recognition have been performed on *P. dominulus* (Dani *et al.* 2001, Lorenzi *et al.* 2004b), *Vespa crabro* (Ruther *et al.* 2002), and honeybees (Dani *et al.* 2005). This research was conducted by supplementing workers with synthetic compounds that are naturally present on the cuticle, and evaluating the response by nestmates. A similar experiment was performed on *Camponotus vagus* (Meskali *et al.* 1995), but with a compound not naturally present on the cuticle of this ant.

In *P. dominulus*, if the worker's cuticle was supplemented with single *n*-alkanes, they were not attacked by their nestmates when released close to their natal nest (Dani *et al.* 2001). This result was also found when wasps were treated with a mixture of *n*-alkanes, which changed their natural profile considerably. Alternatively, treatment with either monomethyl alkanes or alkenes induced aggression by nestmates.

In contrast to Dani *et al.* (2001), Ruther *et al.* (2002) and Lorenzi *et al.* (2004) observed aggression when they applied *n*-alkanes respectively to dead *Vespa crabro* workers, which were then placed close to the nest entrance, or to recently emerged *P. dominulus* females. In this later experiment, wasps were placed on a piece of filter paper treated with the alkane, a situation that approximates the condition of a wasp resting or walking on the nest. Interestingly, Lorenzi *et al.* (2004) replicated the results obtained by Dani *et al.* (2001) when they performed this experiment on individuals older than 24 hours. Similar to what was already demonstrated in *P. fuscatus* (Panek *et al.* 2001), Lorenzi *et al.* (2004) found that in the first three days of life, wasps undergo both an increase in the total amount of hydrocarbons and a change in the major characteristics of their composition, with the relative abundance of methylalkanes and longer chain compounds increasing.

Lorenzi *et al.* (2004) proposed that recently emerged wasps may incorporate exogenous compounds more easily into their cuticle lipids. They suggested this difference is linked to the different cuticular hydrocarbon blends in newly emerged and older wasps, with the higher abundance of shorter chained hydrocarbons and alkenes rendering the cuticular lipids of young wasps more liquid than in older wasps. However they did not consider that many branched alkanes, and in particular dimethylalkanes and centrally branched monomethylalkanes, which are present in high concentration in older wasps, are generally liquid at room temperature (Gibbs & Pomonis 1995). In fact, as noticed by Gibbs (2002), chain length has relatively minor effects on melting temperature, while differences in hydrocarbon classes (alkenes, methylalkanes, etc.) are more important.

A recent paper on three *Pachycondyla* ant species (Lucas *et al.* 2005), analysed quantitative differences in two behaviours that precede fighting. From *P. villosa* and *P. inversa* specimens, the entire body hydrocarbon extract, the non-hydrocarbon fraction, and three different fractions of cuticular hydrocarbons containing *n*-alkanes, alkenes, and methyl alkanes were applied to a filter paper cone and presented to *P. subversa*. The time spent by the ants performing the behaviours that precede fighting was found to differ from the control (solvent) only when the entire hydrocarbon extracts or the methylalkane fraction was used.

A similar experiment was performed by Dani *et al.* (2005) on honeybees, by adding to live foragers the alkene or the alkane fraction (mainly constituted by *n*-alkanes) prepared from body extracts of individuals belonging to alien colonies. The chemically enhanced honey bees were then freed at the entrance of their natal hive. The authors found that the alkene-treated bees, but not the alkane-treated bees, were more frequently attacked by guards than were control bees. Similarly, treatment with single alkenes or mixture of alkenes elicited generally aggressive response by guards, while *n*-alkanes did not.

A great advance in unravelling the "structure" of recognition cues would occur if we understood if all, or only part, of the hydrocarbons present on the cuticle contribute to the recognition signature, and if hydrocarbons with similar structures but with a different chain length (e.g. a series of central monomethylalkanes) are perceived as different compounds. In the absence of such information, the analysis of differences in the epicuticular hydrocarbon composition of conspecifics must consider each compound, and its concentration, as a separate variables. At the same time, some compounds — mainly positional isomers of centrally-branched methylalkanes and positional isomers of dimethylalkanes — are considered single variables because they are currently impossible to separate chromatographically. Although this is the only approach currently available, we must keep in mind that the insect perception of epicuticular hydrocarbon mixtures may differ dramatically from our way of describing their composition.

In honeybees, the capacity to discriminate between different hydrocarbons or hydrocarbon mixtures has been studied using the proboscis extension response (PER) conditioning paradigm (Getz & Smith 1987, Fröhlich *et al*. 2000, 2001, Chaline *et al*. 2005). This approach was previously used to test discrimination between volatile compounds, such as floral odours. Through these experiments, Chaline *et al*. (2005) found differences in the ability to learn and discriminate different cuticular hydrocarbons, with most alkenes being well-learned and highly discriminated. Although the bioassay is very unnatural, these experiments may help in understanding the structure of complex olfactory signals including recognition signatures. Similar conditioning protocols have, to my knowledge, not been developed for other social insects.

Variability of cuticular hydrocarbon mixtures

As noticed by Howard (1993), there is a large amount of variability in insect cuticular hydrocarbons, and this occurs from individual to population levels. Several aspect of the ontogeny of cuticular signature variation has been investigated in *Polistes* wasps. As discussed above, variation of epicuticular hydrocarbon profile was studied in young wasps during the first days after emergence (Panek *et al*. 2001, Lorenzi *et al*. 2004b). Differences between dominant and subordinate foundresses were investigated by Sledge *et al*. (2001) in *P. dominulus*, this research showed that subordinate foundresses develop the same profile as the dominant foundress shortly after the former is removed from the colony.

Dapporto *et al*. (2004b) studied the variation in the profile of overwintering *P. dominulus* foundresses and correlated this with foundresses cooperating in nest foundation. They found that overwintering foundresses originating from different localities could be distinguished on the basis of their epicuticular hydrocarbon composition. However if wasps of two localities hibernated together, their locality identity was obscured by the beginning of spring. Moreover, at the moment of nest foundation, foundresses who hibernated in mixed groups associated with individuals of both localities, while foundresses who hibernated in unmixed groups, but were placed in mixed groups at the beginning of spring, only associated with wasps from their own locality.

Differences between *P. dominulus* populations were investigated further by Dapporto *et al*. (2004c). By studying individuals originating from three different localities, Dapporto *et al*. (2004c) failed to find a common signature among dominant foundresses. However, the authors showed that when the whole cuticular hydrocarbon chromatogram was considered, dominant foundresses of all the three populations had a higher abundance of longer-chain compounds than the subordinates.

Dapporto *et al*. (2004a) examined population differences in *P. dominulus* specimens originating from different islands of the Tyrrhenian Sea. Using cluster analysis, authors found that, although specimens originating from the same island did not always grouped together, each of the two major groupings contained individuals from islands known to share common biogeographic characters.

All data presented thus far indicate that there is significant variation in cuticular hydrocarbon signatures within a species. Although both the importance of cuticular hydrocarbons as nestmate recognition cues, and their within-colony variability, has been documented, few studies simultaneously consider both these aspects. Broader experimental designs are needed to understand if cuticular hydrocarbons guarantee colony identity throughout the colony cycle. *Polistes* colonies, because of their small size, seem a suitable subject to pursue such a study.

Perception of cuticular hydrocarbons

As Howard (1993) noted, although behavioural data strongly indicate that insects perceive cuticular hydrocarbons, the mechanisms by which this is accomplished is poorly understood. Many advances in the understanding of the mechanisms underlying olfactory reception have recently been made using electrophysiological, biochemical, and molecular methods (Jacquin-Joly & Merlin 2004, *see* also Blomquist & Vogt 2003). However, only a few studies were conducted on social insects.

The use of electro-antennography (EAG) or gas chromatography coupled to electro-antennographic detection (EAD), which is widely used for more volatile semiochemicals, has rarely been used in studies of long-chain hydrocarbons in insects. This may be due to technical difficulties. In fact, in the case of EAG, difficulty exists in conveying the compounds to the insect antenna through a room-temperature air flow. In GC-EAD it may be difficult to find conditions by which the antenna can be maintained in a live state when subjected to the high temperature air flow needed to elute long-chain hydrocarbons. Only a few studies used EAG or GC-EAD to test the response of social insects to cuticular hydrocarbons (Weston *et al*. 1997 Batista-Pereira *et al*. 2004, D'Ettorre *et al*. 2004,). Indeed, only one study reported single-sensillum response to cuticular hydrocarbons (Ozaki *et al*. 2005). Only brief mention in a paper reporting electrophysiological and behavioural response to possible alarm substances was given to an EAG experiment testing *Vespula vulgaris* body extracts (Weston *et al*. 1997).

Epicuticular hydrocarbons, mainly consisting of linear alkanes, have been tested using EAG on the termite *Heterotermes tenuis* (Batista-Pereira *et al*. 2004). This research showed that epicuticular hydrocarbons elicited a response significantly different from controls. D'Ettorre *et al*. (2004) used GC-EAD and EAG to test the antennae of workers of the ponerine ant *Pachycondyla inversa*. This research showed an antennal response to 3,11-dimethylheptacosane, a compound found in high abundance on mature queens. Differences from the control (pentane used to dissolve the tested compound) were observed when 100 ng of this dimethylalkane were tested in the EAG experiments, but not for lower and higher quantities. Interestingly, the whole queen extract elicited an EAG response similar to that of the dimethylalkane.

Electrophysiological recordings from single antennal sensilla have recently been reported for the ant *Camponutus japonicus* (Ozaki *et al*. 2005). These authors showed the existence of a particular class of sensillum that respond to nestmate cuticular extracts suspended in water containing either a surfactant or the homospecific recombinant chemosensory protein isolated from the antennae (CjapCSP). The same sensillum did not respond when the extracts were prepared from non-nestmates, and this suggests the existence of a peripheral recognition mechanism allowing detection of colony specific chemical signals, although the physiological basis of this mechanism is unknown. This paper also reports that an aqueous solution of the chemosensory protein (CSP) dissolves homospecific cuticular hydrocarbons, suggesting that this protein may act as a carrier for these substances within the sensillar lymph.

CSPs have been identified in a number of insect species, and within the same species they have been isolated both in chemosensory organs and in non-sensory tissues (Picimbon 2003, Pelosi *et al*. 2005). Similarly to Odorant Binding Proteins (OBPs) and Pheromone Binding Proteins (PBPs), from which they differ in several structural characteristics (Ishida *et al*. 2002, Lartigue *et al*. 2002, Picimbon 2003), CSP are secreted in the lymph of chemosensilla — where they can reach millimolar concentrations. OBPs and PBPs are involved in perireceptor chemoreception acting as carriers for hydrophobic odorant molecules through the sensillum haemolymph to the odorant neuron membrane receptors and/or delivering these molecules to degradative enzymes (Picimbon 2003). Similarly it has been suggested that CSPs may mediate the transport of compounds containing carbon chains (Picimbon 2003), but only one paper has reported evidence to support this (Lartigue *et al*. 2002). As such, the results of Ozaki *et al*. (2005) on *Camponutus japonicus* are of particular interest with respect to the perireceptor events leading to perception of cuticular hydrocarbons and the role of sensillar CSPs.

CSPs and OBPs have recently been studied in *P. dominulus* (Calvello *et al.* 2003, 2005) and *Vespa crabro* (Calvello *et al.* 2005). In both species, CSPs are only expressed in the antennae, while OBPs are expressed in other body parts (legs and wings). Specificity of a CSP to the antennae has also been reported for the ant *Linepithema humile* (Ishida *et al.* 2002). In the honeybee, for which the expression of three CSPs and five OBPs were analysed (Calvello *et al.* 2005), the pattern is complex, showing that for both the classes, some proteins are only expressed in the antennae and others are expressed in other parts of the body.

When the *V. crabro* CSP amino acid sequence was aligned with that of *P. dominulus* and *A. mellifera* (named ASP3c), it was found that the two wasps shared 35% of residues, while *V. crabro* and *A. mellifera* shared 60% (Calvello *et al.* 2005). Moreover, *P. dominulus* shared a higher percent of residues with the CSP amino acid sequence reported for the ants *Linepithema humile* (58%) and *Camponotus japonicus* (63%). Although these results are unexpected and difficult to explain, it is interesting to note that epicuticular lipid composition are much more similar between honeybees and hornets than between this last species and *P. dominulus*. Hornet and honeybee epicuticular lipids are in fact composed mainly of linear alkanes and alkenes in the range from C_{21} to C_{31}, with a few methylalkanes present (Blomquist *et al.* 1980, Francis *et al.* 1985, Butts *et al.* 1991) and some linear fatty alcohols (Dani *et al.* 2004a, 2004b), while *P. dominulus* present alkenes in very low amounts and methylalkanes as major components (Bonavita-Cougourdan *et al.* 1991). Indeed, *P. dominulus* epicuticular hydrocarbons are more similar to those of the two ant species, and in particular to those of *L. humile*, for the high number of methylalkanes present (Liang *et al.* 2001, de Biseau *et al.* 2004, Ozaki *et al.* 2005)

Conclusion

Although studies on epicuticular hydrocarbons as semiochemicals in social insects have produced significant evidence that epicuticular lipids are mediators of complex behaviour in social insects, we currently have very limited knowledge on how such complex mixtures of compounds are perceived. This limited knowledge, particularly evident in comparison with what is known about other insects (for example perception of sexual pheromones in moths), probably derives from the fact that insect social behaviour — and not insect physiology — is the main focus of interest of the researchers involved in Polistes research. In my view, a more interdisciplinary approach using electrophysiological, biochemical, and molecular methods and techniques — already developed for the study of pheromone perception — is strongly needed in the future study of social insect pheromones and of cuticular lipids.

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