JOURNAL OF ZOOLOGICAL SYSTEMATICS AND EVOLUTIONARY RESEARCH

Accepted on 10 June 2009

J Zool Syst Evol Res doi: 10.1111/j.1439-0469.2009.00543.x

[1]*Università degli Studi di Firenze, Dipartimento di Biologia Evoluzionistica "Leo Pardi", Via Romana, Firenze, Italy;* [2]*Istituto Comprensivo Materna Elementare Media Convenevole da Prato via 1° Maggio, Prato, Italy;* [3]*Institute of Biological Sciences, University of Malaya, Kuala Lumpur, Malaysia;* [4]*Centro Interdipartimentale di Spettrometria di Massa dell'Universita' di Firenze, Viale G. Pieraccini, Firenze, Italy*

Medium molecular weight polar substances of the cuticle as tools in the study of the taxonomy, systematics and chemical ecology of tropical hover wasps (Hymenoptera: Stenogastrinae)

David Baracchi[1], Leonardo Dapporto[2], Serafino Teseo[1], Rosli Hashim[3] and Stefano Turillazzi[1,4]

Abstract

The Stenogastrinae wasps have been proposed as a key group for an understanding of social evolution in insects, but the phylogeny of the group is still under discussion. The use of chemical characters, in particular cuticular hydrocarbons, for insect taxonomy is relatively recent and only a few studies have been conducted on the cuticular polar substances. In this work, we ascertain, by the matrix-assisted laser desorption ionization-time of flight mass spectrometry technique, that different species of primitively eusocial hover wasps have different compositions of the epicuticular polar compounds ranging from 900 to 3600 Da. General linear model analysis and discriminant analysis showed that the average spectral profiles of this fraction can be diagnostic for identification of the species. Moreover, for the first time we show population diversification in the medium MW polar cuticular mixtures in insects. In conclusion, the results demonstrate that the chemical characters are consistent with the physical characters and the study support the importance of medium MW polar substances as powerful tools for systematics (chemosystematics) and chemical ecology (fertility signal and population characterization) in a primitively social insect taxon.

Key words: Chemotaxonomy – epicuticular profiling – polar compounds – MALDI-TOF

Introduction

The Stenogastrinae wasps (Hymenoptera, Vespidae) are a taxon of approximately 58 described species in seven genera inhabiting the forests of South-east Asia from South India to New Guinea and from Vietnam to Indonesia (Carpenter and Starr 2000). The largest genera are *Eustenogaster* van der Vecht, 1969, *Liostenogaster* van der Vecht, 1969 and *Parischnogaster* von Schulthess, 1914 all distributed in the Oriental Region. These wasps, owing to their small colonies and primitive social organization, have been proposed as a key group for an understanding of origin and social evolution in insects (Yoshikawa et al. 1969; West-Eberhard 1978; Turillazzi and Pardi 1982; Turillazzi 1991), and several of their features are considered important in promoting or limiting their social evolution [e.g. poor quality of nest material, (Hansell 1987); elaborate larval rearing, (Turillazzi 1989); ensured fitness returns and long larval developmental times, (Field et al. 2000)].

The phylogeny of the group is not completely clear: the prevailing view (Carpenter 1982, 1988) is that the three subfamilies of social wasps form a monophyletic group with the hover wasps as a sister group of the other two social vespid subfamilies Polistinae and Vespinae. However, a recent study by Hines et al. (2007), comparing mitochondrial and nuclear DNA, indicated a clade composed of these three subfamilies as non-monophyletic and implied a separate route to eusociality for the Stenogastrinae. Hence, the systematics of the group is not yet complete: 58 species in seven genera are currently recognized (Carpenter 2001). The genus *Eustenogaster* van der Vecht was recently revised (Saito and Kojima 2007), but the genera with the highest number of species (*Liostenogaster* van der Vecht, 1969, and *Parischnogaster* von Schulthess, 1914) still lack a published revision. Classic taxonomic methods based on differences in morphological character sets continue to be powerful tools for the discrimination of species. Nevertheless, in the Stenogastrinae, characters of nest architecture have also been used to discriminate among very similar species (Sakagami and Yoshikawa 1968). The use of chemical characters for insect taxonomy is relatively recent. In particular, the cuticular hydrocarbon composition has been assessed as a possible chemotaxonomic character in several taxa, such as mosquitoes (Carlson et al. 1997), beetles (Page et al. 1997), grasshoppers (Chapman et al. 1995), butterflies (Dapporto 2007) and, among social insects, ants (Vander Meer 1986), bees (Blum et al. 2000) and termites (Haverty et al. 2000, 2005). Besides having genetic determinants, these compounds are also used in communication and thus may represent a interesting link between DNA and ecological and behavioural traits typical of each species (see Dapporto et al. 2004a,b,c for social wasps). However, hydrocarbons are not the exclusive components of the superficial cuticular layer of insects and more precisely of wasps. In fact, mass spectrometry analysis of cuticular methanol extracts from *Polistes* paper wasps showed a complex pattern of polar substances (Dapporto et al. 2008), including substances similar in part to those found in the medium molecular weight fraction of venoms of various species (Turillazzi et al. 2007). Moreover, it has been also demonstrated that cuticular hydrocarbons may derive directly from prey and thus may be strongly dependent on environmental factors (Liang and Silverman 2000). Conversely, venom and cuticular peptides are likely produced directly by protein synthesis and may represent a more stable indication of phylogenetic relationships.

The aims of the present study were to ascertain whether medium molecular weight polar compounds are present on the

Corresponding author: David Baracchi (biodav82@libero.it)
Authors' email addresses: Leonardo Dapporto (leondap@gmail.com), Serafino Teseo (serafinoteseo@yahoo.it), Rosli Hashim (roslihashim@um.edu.my) and Stefano Turillazzi (stefano.turilla@unifi.it)

cuticle of hover wasps and, if so, whether they have a species-specific composition and could possibly be used to discriminate between different taxa. We used the matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) technique to determine the reference spectra (profiling) of the cuticular layer of some species belonging to three genera of Stenogastrinae. Peptide mapping and spectra profiling using MALDI-TOF MS analysis and other MS techniques have already been used to demonstrate differences between individuals belonging to the same species (Jakubowski et al. 2005) or to establish the phylogenetic relationships among various species belonging to the same taxonomic group (Serrao Wermelinger et al. 2005). In social Vespidae, MS techniques have been employed for the study of medium MW venom fractions from some species of social wasps (Turillazzi et al. 2007) and for the characterization of the main allergens of the venoms of various species (Kolarich et al. 2005). Recently, our group used GC–MS techniques to characterize volatile hydrocarbons and other apolar compounds of the venom of *Polistes* wasps (Bruschini et al. 2006a,b, 2007). The MALDI technique requires very low amounts of substance, allowing us to perform analyses, in a very short time, of the epicuticular polar substances of single individuals belonging to each examined species. In this paper, we report the results obtained from analyses of epicuticular polar odours ranging from 900 to 3000 Da collected from 11 tropical hover wasp species belonging to the three large genera of this subfamily.

Materials and Methods

Species analyses

The subfamily Stenogastrinae, whose distribution is limited to Southeast Asia and Papuan regions, includes species with a primitive social organization. The largest genera are *Eustenogaster, Liostenogaster and Parischnogaster*. The cuticular compounds of the following stenogastrine species were analysed for medium molecular weight polar chemicals: *Eustenogaster calyptodoma* (Sakagami and Yoshikawa 1968), *Eustenogaster micans* (de Saussure, 1852), *Liostenogaster campanulae* (Turillazzi, 1999), *Liostenogaster flavolineata* (Cameron, 1902), *Liostenogaster topographica* (Turillazzi, 1999), *Liostenogaster vechti* (Turillazzi, 1988), *Parischnogaster alternata* (Sakagami, 1969), *Parischnogaster mellyi* (de Saussure, 1852), *Parischnogaster jacobsoni* (du Buysson, 1913), *P.* sp, *Parischnogaster striatula* (du Buysson,1905). All samples were collected in Pahang State in peninsular Malaysia during February–March 2008 (Table S1). As nest architecture is important for species identification, we collected specimens directly on the comb. Since male wasps disperse early after emergence, we focused on females. Some specimens were collected at various sites on Fraser's Hill (locality E–F), others at various localities around Kuala Lumpur: Ulu Langat (locality G), Ulu Gombak (locality B), Genting Sempah (locality C), Janda Baik (locality A) and Genting Highland (locality D). We collected 12 females of each species from 12 different colonies and we killed them by freezing soon after capture. Each wasp was later rinsed in 300 µl of methanol for 3 min to extract the epicuticular polar compounds. Finally, we dissected all the females to determine the stage of ovarian development, measured as the average length of the six largest oocytes present in the ovarioles using a micrometer eye piece.

Mass spectrometry analyses

The matrix for MALDI-TOF experiments was a solution of HCCA (10 mg ml^{-1}) in acetonitrile and 0.1% TFA in water (1 : 1, v:v). A 1 µl volume of the peptide solution was mixed with MALDI matrix (1 : 1, v:v), and the mixture was transferred to a stainless steel target; the droplet was allowed to evaporate before the target was introduced into the mass spectrometer. The stainless steel target, prepared with all the samples, was analysed using an automatic procedure available on the MALDI mass spectrometer to standardize the results.

Methanol extracts from each individual wasp were analysed with a MALDI Ultraflex TOF/TOF (Bruker Daltonics, Bremen, Germany). The instrument was operated in positive ion reflector mode. The accelerating voltage and the Ion Source 2 were set to 25.0 and 21.9 kV, respectively, and the delay time was 20 ns; 800 shots were automatically accumulated for each spectrum. External calibration was performed using the Bruker Standard Peptide Calibration kit (Bruker Daltonics, Bremen, Germany).

We also performed an internal calibration using the FLEX ANALYSIS software by Bruker Daltonics, Bremer, Germany, as we had the exact mass of two peptides (1854 and 1909 Da) identified in the venom of the paper wasp *Polistes dominulus*. Calibrated spectra were imported into the CLINPROTOOLS™ (CPT) software (Bruker Daltonics, Bremen, Germany) and processed with a procedure suggested by the software manual and similar to that used by Zhang et al. (2004) for profiling potential biomarkers in the plasma of asthma patients and to that of a previous study in our laboratory on cuticular and venom peptides (Turillazzi et al. 2006). The program calculates the areas of the most important peaks that may account for statistical differences between various species. This data set was used for statistical analyses using SPSS 13 for Windows (SSPS, Inc., Chicago, IL, USA).

Statistical analyses

We first determined the percentage of each peak area with respect to the total of all peaks and then we log transformed it. We performed a principal components analysis (PCA) to reduce the number of variables subsequently used in general linear model (GLM) analysis and discriminant analysis (DA). Exclusion of variables was based on the anti-image matrix value of each variable. Those lower than 0.2 were excluded and subsequently included as independent variables in GLM and DA. We varimax-rotated the components. The Kaiser–Meyer–Olkin (KMO) test was used to evaluate the sampling adequacy.

To identify which compounds are important for discrimination, a multivariate GLM was performed. Species, ovary index and provenience were used as independent variables and the percentages of each compound as dependent variables. The importance of peaks for discrimination was evaluated in terms of the relative magnitude of *F*-values.

We randomly selected two specimens from each species sample as a cross-validation test sample and used the other 10 specimens per species to create a stepwise discriminant function model aimed at verifying whether the predefined group (species) could be discriminated on the basis of the chemical profiles obtained by MALDI-TOF analysis. DA was performed on the reduced set of factor scores obtained from PCA so as to obtain a more conservative analysis performed on a smaller number of predictor variables. The significance of Wilks' lambda and the percentage of correct assignments were used to estimate the validity of the discriminant functions. Moreover, using the same discriminant functions, we classified the cross-validation specimens inserted in the analysis as unnamed cases. The percentage of blind cases correctly assigned to their species was used as a definitive indication of the validity of the analysis in assigning specimens to their species on the basis of chemical composition. Finally, a cluster analysis, using the average linkage (between group) method and squared Euclidean distance among chemicals as a dissimilarity measure, was used to group the samples in a tree.

Results

We obtained useful chemical samples from 92 animals out of 131; 14 did not give good spectra and 17 had spectra which could not be calibrated by CPT. With a signal-to-noise threshold of 4.0 (on average spectrum) and a relative threshold base peak of 0.001, the software found 62 peaks, with molecular weight ranging from 1040 to 3522 Da (as $z = 1$, values of m/z are reported in equivalent Daltons) (Table S2 and Fig. 1). At present, we have no information about the

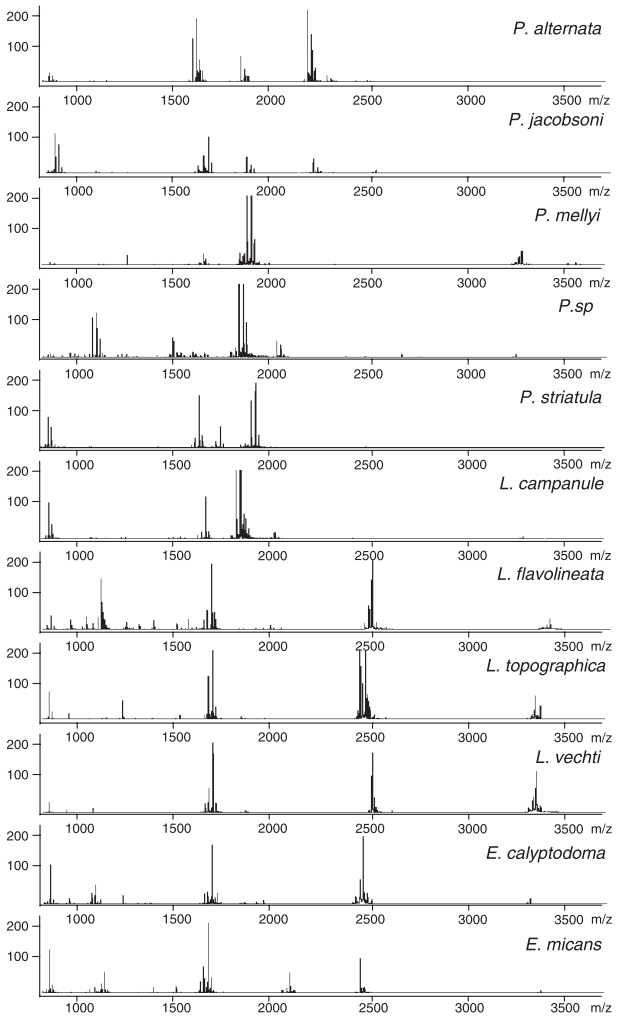

Fig. 1. Average spectra of epicuticular samples from the 11 species (12 samples each) belonging to three genera, *Parischnogaster*, *Liostenogaster* and *Eustenogaster*, obtained with the Centricon-based approach

chemical nature of the detected compounds and their source. Preliminary analyses performed on the venom of some species indicated that most of the compounds found on the cuticle and extracted in methanol came from that secretion, as occurs in *Polistes* wasps (Turillazzi et al. 2006).

After two peaks (corresponding to 1490 and 2075 Da) that did not greatly contribute to the correlation matrix were removed, the PCA extracted 15 PCs (Table S3) that explained 88.10% of the total variance with a KMO of 0.523. A multivariate GLM performed on the whole data set showed that the species strongly differ in their epicuticular profiles. All the 15 PCs generated by PCA showed significant differences except PC3 and PC14 (see Table S4). Conversely, ovarian development influenced the presence of a few chemical compounds, mainly PC7 ($F = 11.968$, $p = 0.01$) and PC8 ($F = 6.386$, $p = 0.014$) (Table S4). However, an in-depth analysis revealed that ovarian development affected medium MW compounds (1624, 1731, 1887 and 1909 represented in PC7) only in *L. topographica* (Fig. 2). After *L. topographica* was removed from the GLM analysis, ovarian development lost any effect on the epicuticular profiling of the other 10 species. Several components (PC2, PC6, PC8, PC11 and PC12) also showed some influence of wasp provenience (Table S4).

Discriminant analysis, using the PC scores and the two compounds not entered in the PCA as species predictors, correctly assigned 93% of the females to their species (Function 1: Wilks' $\lambda = 0.121$, $p < 0.000$ explained variance 48.71%; Function 2: Wilks' $\lambda = 0.023$, $p < 0.000$ explained variance 22.4%, Fig. 3). In particular, all specimens were correctly discriminated except those of *L. campanulae* (confused at 50% with *L. flavolineata* and vice versa) and one individual of *Parischnogaster* sp. (confused with *L. flavolineata*). In the cross-validation sample, only three of 22 cases were misclassified: one *Parischnogaster* sp. individual was classified as *P. jacobsoni* and two *L. topographica* as *E. calyptodoma*.

Cluster analysis, performed by average linkage between groups and using squared Euclidean distance, provided a dendrogram in which relative distances among the 11 species of Stenogastrinae were determined by the distances among their chemical profiles (Fig. 4). In this analysis, the grouping into genera is very good, except for *L. topographica* which was erroneously placed with *Eustenogaster* species. In this dendogram, distances among different taxa overlapped quite well with those obtained by cladistic studies (Carpenter 1988), even if the cluster analysis groups *Eustenogaster* and *Liostenogaster*, while in the cladogram *Liostenogaster* is the sister-group of all other genera.

Discussion

The present study shows that different species of primitively eusocial hover wasps have different compositions of the epicuticular compounds ranging from 900 to 3600 Da MW. Since the main differences appear to be closely related to species, as suggested by the high number of discriminatory peaks and the high *F*-values in the GLM analysis, we can

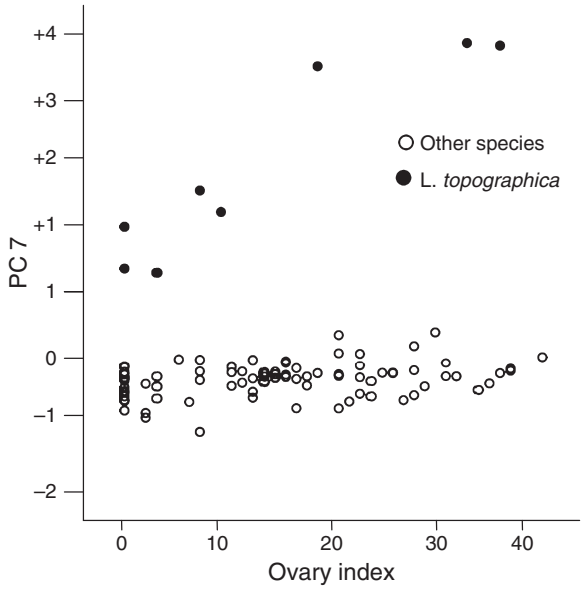

Fig. 2. Correlation between the ovarian development of *L. topographica* (squares) samples and PC7 (representing the medium MW compounds 1624, 1731, 1887 and 1909). The plot also shows that the ovarian development of the other 10 species (circles) had no effect on the compounds represented in PC7

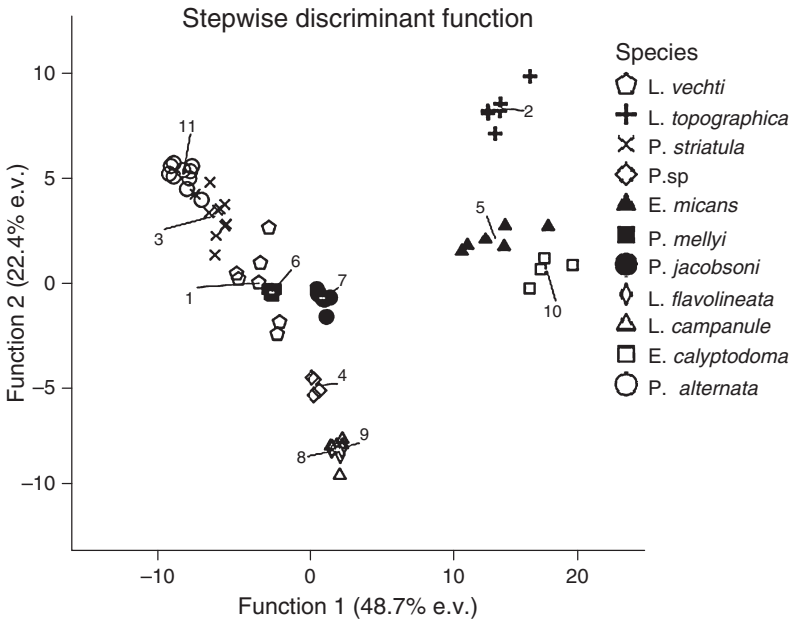

Fig. 3. Stepwise discriminant analysis of female epicuticular compounds of 11 stenogastrine species using PCs provided by PCA

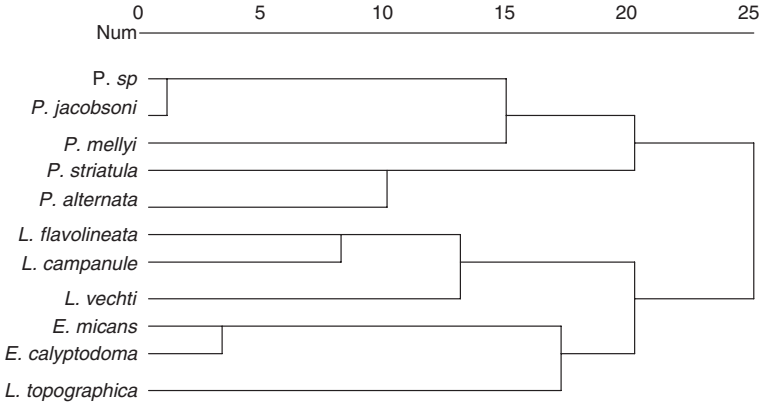

Fig. 4. Dendrogram for some stenogastrine species resulting from cluster analysis performed by average linkage between groups and using squared Euclidean distance. The relative distances among the 11 species were determined by the distances among their chemical profiles

conclude that average spectral profiles of this fraction can be diagnostic for species identification. Indeed, most of the species were perfectly identified and unnamed specimens were all assigned to their correct group. However, the model was unable to correctly discriminate between *L. campanulae* and *L. flavolineata*, probably due to their very close phylogenetic relationship. Moreover, *L. topographica* was grouped near *Eustenogaster* species rather than other species of its genus. This erroneous classification may be as simple as the fact that the clustering method employed was not phylogenetic, it was phenetic, which may not necessarily show phylogenetic relationship. Moreover, *L. topographica* presents some peculiarities with respect to other species of *Liostenogaster*; for example, it builds a quite particular nest and has the largest colonies in terms of individuals of all the hover wasps.

Compositional differences were also found in the hydrocarbons present in the venom sacs of seven species of Stenogastrinae wasps (Dani et al. 1998), although the data were not subjected to multivariate statistics. However, proteinaceous components like medium MW compounds, as we emphasized in the Introduction, are probably more precise tools for systematics than hydrocarbons (Turillazzi et al. 2007).

Nevertheless, GLM analysis showed small differences between populations collected at different sites, suggesting a minor influence of some ecological determinants, such as food or materials used to construct the nest. Such differences in cuticular hydrocarbons have been highlighted among populations of *Polistes* paper wasps (Dapporto et al. 2004a,b,c). The differences among the Stenogastrinae populations, in our study, are the first evidence of population diversification in medium MW polar cuticular mixtures in insects. It is not known whether hover wasps perceive and respond to geographical diversification of cuticular mixtures, although the perception of population differences could be one of the mechanisms that allow differential mate-choice and speciation (Gemeno et al. 2000; Krokos et al. 2002; Dapporto et al. 2004a; Dapporto 2007).

Finally, the degree of ovarian development seems to have had no influence on the epicuticular profiles of all investigated species except *L. topographica* (whereas it seems to influence cuticular hydrocarbons in some of the same species, cf. Turillazzi et al. 2004). In *L. topographica*, the amounts of four main compounds of the cuticular polar profile showed a clear correlation with ovarian development, suggesting that these

substances function as a fertility signal in this species. Yet, the presence of a fertility signal in Stenogastrinae is unexpected, since these wasps have a simple social structure and the number of females within the colonies is low compared with other social wasps. In species with a similar social structure, direct physical interactions, rather than pheromonal communication, are expected to act as social regulators (Jeanne 1991). Moreover, individuals with developed ovaries form a high percentage of colonial populations of various stenogastrine species, and this is a sign of primitive social organization (Turillazzi 1991). However, as we already pointed out, *L. topographica* is the species with the highest number of females living together on the nest among all Stenogastrinae wasps (Baracchi et al. 2009), and thus the possible presence of a fertility signal in this species is intriguing. Although no specific studies have been carried out to verify whether insects are able to use polar cuticular compounds in communication, a caste-specific profile was demonstrated in *P. dominulus* (Dapporto et al. 2008) and the existence of proteinaceous polar pheromones to identify queen-laid larvae from worker-laid ones has been suggested in fire ants (Klobuchar and Deslippe 2002). Further observations and behavioural experiments on *L. topographica* are necessary to ascertain the genetic structure of the colonies and to determine if cuticular compounds constitute a fertility signal in this species.

In conclusion, the results demonstrate that the chemical characters are consistent with the physical characters and the study support the importance of medium MW polar substances as powerful tools for systematics (chemosystematics) and chemical ecology (fertility signal and population characterization) in a primitively social insect taxon.

Acknowledgements

The authors thank Duccio Lambardi (CISM, University of Florence) for his help in chemical analyses. We thank Bruker Daltonics for the supply of CPT software. The authors also thank Ente Cassa di Risparmio di Firenze for funding the acquisition of the MALDI-TOF/TOF. Financial support was provided by Italian Ministry for University and Research, PRIN 2006 project 'Identification and expression analysis of proteins involved in chemical communication in three model insects using innovative mass spectrometry techniques'.

Riassunto

I composti polari epicuticolari di medio peso molecolare come strumento d'indagine nella tassonomia, nella sistematica e nell'ecologia chimica delle vespe Stenogastrinae

Le vespe primitivamente eusociali della sottofamiglia delle Stenogastrinae sono state proposte come gruppo chiave per lo studio dell'evoluzione della socialità negli insetti. Ancora oggi, però, la filogenesi del gruppo rimane incerta e discussa. Sebbene l'uso dei caratteri chimici, e in particolare degli idrocarburi cuticolari, negli studi tassonomici sugli insetti sia ben noto, seppur di recente applicazione, sono ancora pochi gli studi condotti sulle sostanze cuticolari di natura polare. In questo lavoro abbiamo saggiato, mediante tecniche di spettrometria di massa (MALDI-TOF MS), la presenza di composti polari cuticolari di massa compresa tra i 900 e i 3600 (probabilmente provenienti dal veleno) sulla cuticola di alcune specie di vespe stenogastrine. Analisi statistiche multivariate hanno dimostrato che lo spettro medio dei profili chimici nel range da noi indagato può essere utilizzato per l'identificazione delle specie e la nostra ricerca propone questo metodo come un potente strumento non solo per studi di tassonomia (chemotassonomia) ma anche per studi di sistematica e di ecologia chimica (es. segnali di fertilità e differenze intra-popolazioni) nei taxon di insetti primitivamente eusociali.

References

Baracchi D, Hashim R, Teseo S, Turillazzi S (2009) Basic social biology and nest architecture of *Liostenogaster topographica* Turillazzi 1999 (Hymenoptera Stenogastrinae). Trop Zool **22:**15–25.

Blum MS, Fales HM, Morse RA, Underwood BA (2000) Chemical characters of two related species of giant honeybees (*Apis dorsata* and *A. laboriosa*): possible ecological significance. J Chem Ecol **26:**801–807.

Bruschini C, Dani FR, Pieraccini G, Guarna F, Turillazzi S (2006a) Volatiles from the venom of five species of paper wasps (*Polistes dominulus*, *P. gallicus*, *P. nimphus*, *P. sulcifer* and *P. olivaceus*). Toxicon **47:**812–825.

Bruschini C, Dani FR, Pieraccini G, Guarna F, Turillazzi S (2006b) Erratum to "Volatiles from the venom of five species of paper wasps (*Polistes dominulus*, *P. gallicus*, *P. nimphus*, *P. sulcifer* and *P. olivaceus*)". Toxicon **48:**473.

Bruschini C, Cervo R, Dani FR, Turillazzi S (2007) Can venom volatiles be a taxonomic tool for *Polistes* wasps (Hymenoptera, Vespidae)?. J Zool Syst Evol Res **45:**202–205.

Carlson DA, Reinert JF, Bernier UR, Sutton BD, Seawright JA (1997) An analysis of the cuticular hydrocarbons among species of the *Anopheles quadrimaculatus* complex (Diptera: Culicidae). J Am Mosquito Control **13:**103–111.

Carpenter JM (1982) The phylogenetic relationships and natural classification of the Vespoidea (Hymenoptera). Syst Entomol **7:**11–38.

Carpenter JM (1988) The phylogenetic system of the Stenogastrine (Hymenoptera: Vespidae). J New York Entomol Soc **96:**140–175.

Carpenter JM (2001) New generic synonymy in Stenogastrinae (Insecta: Hymenoptera; Vespidae). Nat Hist Bull Ibaraki Univ **5:**27–30.

Carpenter JM, Starr CK (2000) A new genus of hover wasps from South east Asia (Hymenoptera: Vespidae; Stenogastrinae). Am Mus Nov **3291:**1–12.

Chapman RF, Espelie KE, Sword GA (1995) Use of cuticular lipids in grasshopper taxonomy: a study of variation in *Schistocerca shoshone* (Thomas). Biochem Syst Ecol **23:**383–398.

Dani FR, Morgan ED, Jones GR, Turillazzi S, Cervo R, Francke W (1998) Species-specific volatile substances in the venom sac of hover wasps. J Chem Ecol **24:**1091–1104.

Dapporto L (2007) Cuticular lipid diversification in *Lasiommata megera* and *L. paramegera*: the influence of species, sex, and population (Lepidoptera, Nymphalidae). Biol J Linn Soc **91:**703–710.

Dapporto L, Pansolli C, Turillazzi S (2004a) Hibernation clustering and its consequences for associative nest foundation in *Polistes dominulus* (Hymenoptera Vespidae). Behav Ecol Sociobiol **56:**315–321.

Dapporto L, Theodora P, Spacchini C, Pieraccini G, Turillazzi S (2004b) Rank and epicuticular hydrocarbons in different populations of the paper wasp *Polistes dominulus* (Christ) (Hymenoptera Vespidae). Insect Soc **51:**279–286.

Dapporto L, Palagi E, Turillazzi S (2004c) Cuticular hydrocarbons of *Polistes dominulus* as a biogeographic tool: a study on some populations of the Tuscan Archipelago and the surrounding areas. J Chem Ecol **30:**2139–2151.

Dapporto L, Lambardi D, Turillazzi S (2008) Not only cuticular lipids: first evidence of differences between foundresses and their daughters in polar substances in the paper wasp *Polistes dominulus*. J Insect Physiol **54:**89–95.

Field JP, Shreeves G, Sumner S, Casiraghi M (2000) Insurance-based advantage to helpers in a tropical hover wasp. Nature **404:**869–871.

Gemeno C, Lutfallah AF, Haynes KF (2000) Pheromone blend variation and cross-attraction among populations of the black cutworm moth (Lepidoptera: Noctuidae). Ann Entomol Soc Am **93:**1322–1328.

Hansell MH (1987) Nest building as a facilitating and limiting factor in the evolution of eusociality in the Hymenoptera. In: Harvey P, Partridge L (eds), Oxford Surveys in Evolutionary Biology. Oxford University Press, U.K, pp 155–181.

Haverty MI, Woodrow RJ, Nelson LJ, Grace JK (2000) Cuticular hydrocarbons of termites of the Hawaiian islands. J Chem Ecol **26:**1167–1191.

Haverty MI, Woodrow RJ, Nelson LJ, Grace JK (2005) Identification of termite species by the hydrocarbons in their feces. J Chem Ecol **31:**2119–2151.

Hines HM, Hunt JH, O'Connor TK, Gillespie JJ, Cameron SA (2007) Multigene phylogeny reveals eusociality evolved twice in vespid wasps. Proc Natl Acad Sci USA **104:**3295–3299.

Jakubowski JA, Kelley WP, Sweedler JV, Gilly WF, Schulz JR (2005) Intraspecific variation of venom injected by fish-hunting *Conus* snails. J Exp Biol **208:**2873–2883.

Jeanne RL (1991) The swarm founding Polistinae. In: Ross KG, Matthews RW (eds), The Social Biology of Wasps. Cornell University Press, New York, pp 191–231.

Klobuchar EA, Deslippe RJ (2002) A queen pheromone induces workers to kill sexual larvae in colonies of the red imported fire ant (*Solenopsis invicta*). Naturwissenschaften **89:**302–304.

Kolarich D, Leonard R, Hemmer W, Altmann F (2005) The N-glycans of yellow jacket venom hyaluronidases and the protein sequence of its major isoform in *Vespula vulgaris*. FEBS J **272:**5182–5190.

Krokos FD, Améline A, Bau J, Sans A, Konstantapoulou M, Frérot B, Guerrero A, Eizaguirre M, Malosse C, Etchepare O, Albajes R, Mazomenos B (2002) Comparative studies of female sex pheromone components and male response of the cron stalk borer *Sesamia nonagrioides* in three different populations. J Chem Ecol **28:**1463–1472.

Liang D, Silverman J (2000) "You are what you eat": diet modifies cuticular hydrocarbons and nestmate recognition in the Argentine ant, *Linepithema humile*. Naturwissenschaften **87:**412–416.

Page M, Nelson LJ, Blomquist GJ, Seybold SJ (1997) Cuticular hydrocarbons as chemotaxonomic characters of pine engraver beetles (*Ips* spp.) in the grandicollis subgeneric group. J Chem Ecol **23:**1053–1099.

Saito F, Kojima JL (2007) A taxonomic revision of the hover wasp genus *Eustenogaster* Van der Vecht (Insecta : Hymenoptera; Vespidae, Stenogastrinae). Zootaxa **1556:**1–30.

Sakagami SF, Yoshikawa K (1968) A new ethospecies of *Stenogaster* wasps from Sarawak, with a comment on the value of ethological characters in animal taxonomy. Annot Zool Japon **41:**77–84.

Serrao Wermelinger L, Dutra Denis LS, Oliveira-Carvalho AL, Soares MR, Bloch C, Zingali RB (2005) Fast analysis of low molecular mass compounds present in snake venom: identification of ten new pyroglutamate-containing peptides. Rapid Commun Mass Spectrom **19:**1703–1708.

Turillazzi S (1989) The origin and evolution of social life in the Stenogastrinae (Hymenoptera Vespidae). J Insect Behav **2:**649–661.

Turillazzi S (1991) The Stenogastrinae. In: Ross KG, Matthews RW (eds), The Social Biology of Wasps. Cornell University Press, New York, pp 74–98.

Turillazzi S, Pardi L (1982) Social behavior of *Parischnogaster nigricans serrei* (Du Buysson) (Hymenoptera Stenogastrinae) in Java. Ann Entomol Soc Am **75:**657–664.

Turillazzi S, Sledge MF, Dapporto L, Landi M, Fanelli D, Fondelli L, Zanetti P, Dani FR (2004) Epicuticular lipids and fertility in primitively social wasps (Hymenoptera Stenogastrinae). Physiol Entomol **29:**1–8.

Turillazzi S, Dapporto L, Pansolli C, Boulay R, Dani FR, Moneti G, Pieraccini G (2006) Habitually used hibernation sites of paper wasps are marked with venom and cuticular peptides. Cur Biol **16:**R530–R531.

Turillazzi S, Bruschini C, Lambardi D, Francese S, Spadolini I, Mastrobuoni G (2007) Comparison of the medium molecular weight venom fractions from five species of common social wasps by MALDI-TOF spectra profiling. J Mass Spectrom **42:**199–205.

Vander Meer RK (1986) Chemical taxonomy as a tool for separating *Solenopsis* spp. In: Lofgren CS, Vander Meer RK (eds), Fire Ants and Leaf Cutting Ants: Biology and Management. Westview Press, Boulder, pp 316–326.

West-Eberhard MJ (1978) Polygyny and the evolution of social behavior in wasps. J Kansas Entomol Soc **51:**832–856.

Yoshikawa K, Ohgushi R, Sakagami SF (1969) Preliminary report on entomology of the Osaka City University, 5th Scientific Expedition to Southeast Asia 1966. With descriptions of two new genera of stenogasterine [sic] wasps by J. Van der Vecht. Nat Life Southeast Asia **6:**153–182.

Zhang X, Leung SM, Morris CR, Shigenaga MK (2004) Evaluation of a novel, integrated approach using functionalized magnetic beads, bench-top MALDI-TOF-MS with prestructured sample supports, and pattern recognition software for profiling potential biomarkers in human plasma. J Biomol Tech **15:**167–175.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Number of individuals, number of nests from which they originated, number of individuals of known ovarian development (Ovd) and collection localities for the 11 species investigated.

Table S2. List of the 62 main MALDI-TOF peaks, ranging from 1040 to 3522 *m/z*, identified by the CPT in 11 stenogastrine species. As $z = 1$, values of *m/z* are in equivalent Daltons.

Table S3. Factor loadings (with varimax rotation). In the principal components (PC), only loadings >0.500 are shown (e.v. = explained variance).

Table S4. GLM analysis results: the table reports the 15 principal components (PCs) and their correlation with ovarian development, provenience and species. *F*-values and significance level: *$p < 0.05$; **$p < 0.001$.

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.