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Can venom volatiles be a taxonomic tool for *Polistes* wasps (Hymenoptera, Vespidae)?

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Abstract

In the present study, we investigated whether venom volatiles have a species-specific composition and could possibly be used to discriminate between related paper wasp species. We compared venom volatile chemical profiles, obtained through gas chromatography-mass spectrometry analyses, of three non-parasitic European *Polistes* species (*P. dominulus*, *P. gallicus* and *P. nimphus*). The results show that the venom volatile composition is indeed species specific and can thus be a useful systematic tool.

Key words: *Polistes* – venom volatiles – chemotaxonomy – wasps

Introduction

Classical taxonomic methods based on differences in morphological character sets and on biomolecular features continue to be powerful tools for revealing cryptic species. However, other characters can also be used to discriminate among species. The use of chemical characters for insects taxonomy is relatively recent but well known. In particular, cuticular hydrocarbon composition has been assessed in the past few decades as a possible chemotaxonomic character in several different taxa, such as mosquitoes (Carlson et al. 1997), beetles (Page et al. 1997), grasshoppers (Chapman et al. 1995) and among social insects in ants (Vander Meer 1986), bees (Blum et al. 2000) and termites (Haverty et al. 2000, 2005).

Although several studies have been conducted on cuticular hydrocarbons of *Polistes* social wasps (family Vespidae), all of them were aimed at investigating their function as recognition cues (Gamboa 2004; Dani 2006) in the context of nestmate, caste and rank recognition (Bonavita-Cougourdan et al. 1987; Lorenzi et al. 1996; Sledge et al. 2001, 2004; Dapporto et al. 2005), rather than their possible use as taxonomic features.

Only recently Dapporto et al. (2004) used *P. dominulus* cuticular hydrocarbon composition as a character to investigate differences among populations of different islands of the Tuscan Archipelago.

A recent study carried out by our group showed that venom volatiles of some European species of *Polistes* is different among the species analysed (Bruschini et al. 2006a,b). In the present study we further investigated through discriminant function analysis, whether venom volatiles of three of the most common European species of *Polistes* [*P. dominulus* (Christ, 1791); *P. nimphus* (Christ, 1791) = *P. nimpha* of previous authors, see Carpenter 1996; and *P. gallicus* (Linnaeus, 1767)] are species specific and therefore can be used as taxonomical characters.

Materials and Methods

Venom volatile content analysis

In 2004, worker specimens of three European species belonging to the genus *Polistes*, subgenus *Polistes* sensu stricto (*P. dominulus*, $n = 10$; *P. gallicus*, $n = 11$; *P. nimphus*, $n = 11$) were collected from different locations near Florence (Italy).

After collection, all specimens were immediately stored at −20°C until venom extraction.

The venom was collected from the sting with the aid of a glass capillary. The venom collected from each specimen was stored in a 250-μl glass conical insert, sealed in a 2-ml glass vial and kept at −20°C until analysis.

Volatile components were sampled from the headspace of each vial by means of solid phase micro-extraction (HS-SPME) (Moneti et al. 1997), analysed by gas chromatography-mass spectrometry (GC-MS) and identified as described in Bruschini et al. (2006a).

Statistical analysis

The gas chromatogram obtained for each venom sample was integrated and each peak area was expressed as the percentage of the total area of the peaks present. Discriminant analyses were calculated on the obtained data set (SPSS 13 for Windows). All compounds present in less than 75% of the samples or in less than 75% of the individuals belonging to the same species were excluded from the analysis to reduce the number of variables with a lot of null values for multivariate analysis.

Stepwise discriminant analysis was used to determine whether the predefined groups (species) could be discriminated on the basis of the chemical profiles of their venom volatiles, and which compounds were important for discrimination. The significance of Wilk's lambda and the percentage of correct assignment were used to estimate the validity of the discriminant function.

Results

In Table 1 we reported all the compounds found in the three species, including those excluded for the analysis according to the established criteria (see Materials and Methods). The major compounds found in the venom were identified as spiroacetals, amides, acetates and propanoates of aliphatic secondary alcohols.

We performed a stepwise discriminant analysis on the analysed workers of the three European species (*P. dominulus*, *P. nimphus*, *P. gallicus*) considering 27 among the 46 compounds present. The analysis shows that the workers of these species are totally separated (100% of the individuals were correctly assigned to their original groups), indicating that venom volatile composition is species specific (Fig. 1). 2-nPropyl-8-methyl-1,7-dioxaspiro[5.5]undecane and two unidentified compounds were used by stepwise discriminant

Table 1. All the venom volatile compounds found in the three *Polistes* species are shown (*P. d.* = *P. dominulus*, *P. g.* = *P. gallicus*, *P. n.* = *P. nimphus*). For all the compounds is reported the percentage of individuals of each species in which each compound is present. Compounds marked with an asterisk are not included in the discriminant analysis, whilst those in bold were used to generate the two functions in the discriminant analysis

Compound	*P. d.* ($n = 10$)	*P. g.* ($n = 11$)	*P. n.* ($n = 11$)
Unidentified*	50	0	0
(*E*)-4,8-Dimethyl-1,3,7-nonatriene	90	0	0
6-Methyl-5-hepten-2-yl acetate	100	64	91
(*E*,*E*)-2,8-Dimethyl-1,7-dioxaspiro[5.5]undecane	80	91	0
N-(3-Methylbutyl)acetamide	90	73	100
2-Ethyl-7-methyl-1,6-dioxaspiro[4.5]decane isomer A	80	55	0
2-Ethyl-7-methyl-1,6-dioxaspiro[4.5]decane isomer B*	60	73	0
2-Methyl-7-ethyl-1,6-dioxaspiro[4.5]decane*	20	27	0
Unidentified*	50	0	0
2,8-Dimethyl-1,7-dioxaspiro[5.5]undecane*	40	0	0
N-(3-Methylbutyl)propanamide	10	100	0
Unidentified*	60	10	27
2-Nonanyl acetate	100	100	100
Undecen-2-ol*	30	18	0
2-Undecanone*	10	27	18
2-Undecanol	90	64	18
2-Nonanyl propanoate	30	91	0
2-nPropyl-8-methyl-1,7-dioxaspiro[5.5]undecane	0	91	0
Unidentified*	20	18	0
Unidentified*	40	0	0
Unidentified*	70	0	0
Unidentified	90	0	0
Unidentified	60	0	82
Unidentified	100	0	82
Unidentified*	40	0	0
2-Undecenyl acetate	100	100	100
2-Undecanyl acetate	100	100	100
6,10-Dimethyl-(*E*)-5,9-undecadien-2-one (Geranyl acetone)	100	91	100
2-Undecenyl propanoate	0	100	0
2-Undecanyl propanoate	0	100	0
Unidentified*	20	0	0
Unidentified*	20	0	0
6,10-Dimethyl-(*Z*)-5,9-undecadien-2-yl acetate ((*Z*)-5-Tangerinol)	100	10	55
6,10-Dimethyl-(*E*)-5,9-undecadien-2-yl acetate ((*E*)-5-Tangerinol)	100	100	100
2-Tridecenyl acetate isomer A	100	100	100
2-Tridecenyl acetate isomer B	100	100	100
2-Tridecanyl acetate	100	91	100
6,10-Dimethyl-(*E*)-5,9-undecadien-2-yl propanoate	0	82	0
2-Tridecenyl propanoate isomer A	0	82	0
2-Tridecenyl propanoate isomer B *	0	64	0
2-Tridecanyl propanoate*	0	55	0
Unidentified*	40	0	18
Unidentified*	40	0	18
2-Pentadecanyl acetate	70	82	82
3,7,11-Trimethyl-(*E*)6,(*E*)10-dodecatrien-2-yl acetate ((E,E)-Farnesyl acetate)	80	55	100
2-Pentadecanyl propanoate*	0	10	0

analysis to generate function 1 (explained 76.3% of variance, Wilks' lambda = 0.003, $p < 0.001$), whilst 2-undecanol, 2-undecenyl acetate, geranyl acetone and (E)-5-tangerinol were used by stepwise discriminant analysis to generate function 2 (explained 23.7% of variance, Wilks' lambda = 0.088, $p < 0.001$).

Table 1 also shows that the majority of the compounds not included in the analysis are present only in one or two of the considered species.

Discussion

The present work shows that the three European species of the genus *Polistes* (*P. dominulus*, *P. gallicus*, *P. nimphus*) could be clearly distinguished on the basis of their venom volatile profiles which are therefore species specific.

A similar species specificity was found for the volatile compounds present in the venom sacs of seven species of Stenogastrinae wasps (Dani et al. 1998), although data were not investigated through multivariate statistics.

In many Vespinae and Polistinae species venom volatiles have been found to induce an alarm response (Maschwitz 1964, 1984; Ishay et al. 1965; Saslavasky et al. 1973; Jeanne 1981, 1982; Aldiss 1983; Post et al. 1984; Veith et al. 1984; Landolt and Heath 1987; Moritz and Bürgin 1987; Maschwitz and Hanel 1988; Kojima 1994; Landolt et al. 1995; Sledge et al. 1999; Dani et al. 2000; Ono et al. 2003; Fortunato et al. 2004). Bruschini (2005) and Bruschini et al. (2006c) also showed that in *P. dominulus*, *P. nimphus* and *P. gallicus*, the venom extracted from workers recruits nestmates to attack intruders stimulating an alarm response in the colony. It is possible that closely related species, such as the European *Polistes* analysed in the present work, have evolved different alarm pheromones. However, the venom volatiles of the analysed species (Bruschini et al. 2006a,b) are a complex mixture of compounds, most of which are common to all the

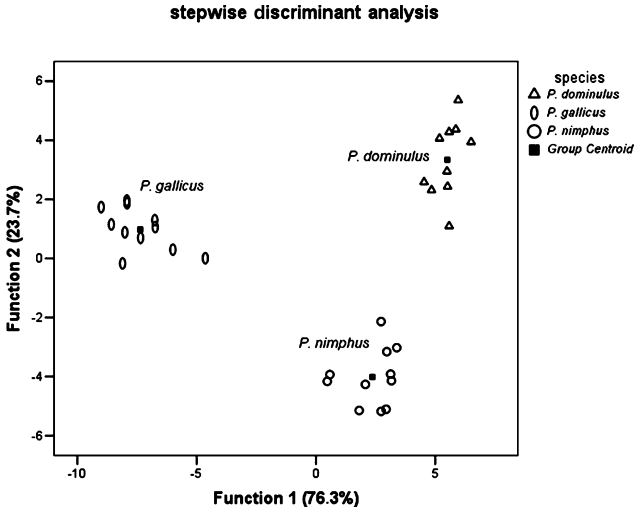

Fig. 1. Stepwise discriminant analysis of worker venom volatiles of the three European species (*Polistes dominulus*, *P. gallicus* and *P. nimphus*)

species, so it can be possible that only one or a few of these compounds may have an alarm function. In *Vespula vulgaris* (Aldiss 1983), *Vespula maculifrons* and *Vespula squamosa* (Landolt et al. 1995), N-3-methylbutylacetamide (MBA) was the major compound found in the venom and, in the latter two species, it was demonstrated to be responsible for alarm response. The same compound has been found, even if in different relative quantities, in all the *Polistes* species so far analysed (Bruschini et al. 2006a,b) but its function as alarm pheromone has not been tested yet.

Independently of the alarm pheromone identification, the present work shows, for the first time, that venom volatiles could be an additional chemotaxonomic method useful to discriminate among aculeate-related species.

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Riassunto

Possono i composti volatili del veleno essere uno strumento tassonomico per le vespe del genere Polistes (Hymenoptera, Vespidae)?

Nel presente lavoro, abbiamo verificato la possibilità di discriminare tre specie di *Polistes* filogeneticamente vicine sulla base della composizione della frazione volatile del veleno. A questo scopo abbiamo confrontato i profili chimici dei composti volatili del veleno, analizzati attraverso GC-MS, di tre specie europee di *Polistes* (*P. dominulus*, *P. gallicus* e *P. nimphus*). I nostri risultati mostrano che tali molecole presenti nel veleno dei *Polistes* Europei sono specie-specifiche. Da questo lavoro emerge che i volatili del veleno potrebbero essere un utile strumento sistematico complementare ai metodi tassonomici tradizionali.

References

Aldiss JBJF (1983) Chemical communication in British social wasps (Hymenoptera: Vespidae). PhD Thesis, University of Southampton, UK.

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.

Bonavita-Cougourdan A, Clément JL, Lange C (1987) Nestmate recognition: The role of cuticular hydrocarbons in the ant *Camponotus vagus* Scop. J Entomol Sci **22:**1–10.

Bruschini C (2005) Difesa della colonia e feromoni di allarme in vespe europee del genere *Polistes* (Hymenoptera, Vespidae). PhD Thesis, University of Florence, Italy.

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:**473–475.

Bruschini C, Cervo R, Turillazzi S (2006c) Evidence of alarm pheromones in the venom of *Polistes dominulus* workers (Hymenoptera: Vespidae). Physiol Entomol **31:**286–293.

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 Contr **13:**103–111.

Carpenter JM (1996) Distributional checklist of species of the genus *Polistes* (Hymenoptera: Vespidae; Polistinae, Polistini). Am Mus Novit **3188:**1–39.

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 (2006) Cuticular lipids as semiochemicals in paper wasps and other social insects. Ann Zool Fennici **43:**500–514.

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.

Dani FR, Jeanne RL, Clarke SR, Jones GR, Morgan ED, Francke W, Turillazzi S (2000) Chemical characterization of the alarm pheromone in the venom of *Polybia occidentalis* and of volatiles from the venom of *P. sericea*. Physiol Entomol **25:**363–369.

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

Dapporto L, Sledge MF, Turillazzi S (2005) Dynamics of cuticular chemical profiles of *Polistes dominulus* workers in orphaned nests. J Insect Physiol **51:**969–973.

Fortunato A, Dani FR, Sledge MF, Fondelli L, Turillazzi S (2004) Alarm communication in *Ropalidia* social wasps. Insect Soc **51:**299–305.

Gamboa GJ (2004) Kin recognition in eusocial wasps. Ann Zool Fennici **41:**789–808.

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.

Ishay J, Ikan R, Bergmann ED (1965) The presence of pheromones in the Oriental hornet, *Vespa orientalis* F. J Insect Physiol **11:**1307–1309.

Jeanne RL (1981) Alarm recruitment, attack behaviour, and the role of alarm pheromones in *Polybia occidentalis* (Hymenoptera: Vespidae). Behav Ecol Sociobiol **9:**143–148.

Jeanne RL (1982) Evidence for an alarm substance in *Polistes canadensis*. Experientia **38:**329–330.

Kojima J (1994) Evidence for an alarm pheromone in the Old World, swarm-founding paper wasp, *Ropalidia romandi* (Le Guillou) (Hymenoptera: Vespidae). J Aust Entomol Soc **33:**45–47

Landolt PJ, Heath RR (1987) Alarm pheromone behavior of *Vespula squamosa* (Hymenopter: Vespidae). Fla Entomol **70:**222–225.

Landolt PJ, Heath RR, Reed HC, Manning K (1995) Pheromonal mediation of alarm in the eastern yellowjacket (Hymenoptera: Vespidae). Fla Entomol **78:**101–108.

Lorenzi MC, Bagnères AG, Clément JL (1996) The role of cuticular hydrocarbons in social insects: is the same in paper wasps? In: Turillazzi S, West-Eberhard MJ (eds), Natural History and Evolution of Paper Wasps. Oxford University Press, Oxford, pp 178–189.

Maschwitz U (1964) Alarm substances and alarm behaviour in social Hymenoptera. Nature **204:**324–327.

Maschwitz U (1984) Alarm pheromone in the long-cheeked wasp *Dolichovespula saxonica* (Hymenoptera: Vespidae). Deut Entomol Z **31:**33–34.

Maschwitz U, Hanel H (1988) Biology of the South East Asian nocturnal wasp, *Provespa anomala*. Entomol Gen **14:**47–52.

Moneti G, Dani FR, Pieraccini G, Turillazzi S (1997) Solidphase microextraction of insect epicuticular hydrocarbons for gas chromatographic/mass spectrometric analysis. Rapid Commun Mass Spectrom **11:**857–862.

Moritz FA, Bürgin H (1987) Group response to alarm pheromones in social wasps and honeybee. Ethology **76:**15–26.

Ono M, Terabe H, Hori H, Sasaki M (2003) Components of giant hornet alarm pheromone. Nature **424:**637–638.

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.

Post DC, Downing HA, Jeanne RL (1984) Alarm response to venom by social wasps *Polistes exclamans* and *Polistes fuscatus*. J Chem Ecol **10:**1425–1433.

Saslavasky H, Ishay J, Ikan R (1973) Alarm substances as toxicants of the oriental hornet, *Vespa orientalis*. Life Sci **12:**135–143.

Sledge MF, Dani FR, Fortunato A, Maschwitz U, Clarke SR, Francescato E, Hashim R, Morgan ED, Jones GR, Turillazzi S (1999) Venom induces alarm behaviour in the social wasp *Polybioides raphigastra* (Hymenoptera: Vespidae): an investigation of alarm behaviour, venom volatiles and sting autotomy. Physiol Entomol **24:**234–239.

Sledge MF, Boscaro F, Turillazzi S (2001) Cuticular hydrocarbon and reproductive status in the social wasp *Polistes dominulus*. Behav Ecol Sociobiol **49:**401–409.

Sledge MF, Trinca I, Massolo A, Boscaro F, Turillazzi S (2004) Variation in cuticular hydrocarbon signatures, hormonal correlates and establishment of reproductive dominance in a polistine wasp. J Insect Physiol **50:**73–83.

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, CO, pp. 316–326.

Veith HJ, Koeniger N, Maschwitz U (1984) 2-Methyl-3-buten-2-ol a major component of the alarm pheromone of the hornet *Vespa crabro*. Naturwissenschaften **71:**328–329.

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