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Polistes venom: a multifunctional secretion

Stefano Turillazzi

Centro di Spettrometria di Massa & Dipartimento di Biologia Animale e Genetica "L. Pardi", Università di Firenze, via Romana 17, I-50125 Firenze, Italy (e-mail: turillazzi@dbag.unifi.it).

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Polistes venom is a complex secretion that has several functions in the social organization of a colony. The defensive function as an allomone against vertebrate and invertebrate enemies is enhanced with antimicrobial properties. Its role in chemical communication is multi-faceted as it contains both alarm and sexual pheromones, but can also have cues which are important both for nestmate and caste recognition, as well as for hibernacula marking. Research on venom chemistry is extremely important for both the establishment of specific immunotherapy for allergic patients and for the discovery of new molecules with pharmacological activity. Venom composition can also provide important characters for taxonomical and phylogenetical studies.

Introduction

Using weapons that evolved for prey capture as defence against enemies of their own brood was one of the major factors that favoured the evolution of social life in Aculeate Hymenoptera (Hermann & Blum 1981, Andersson 1984, Starr 1985). The stinging apparatus and the secretion which it delivers — the venom — became more developed in those species (e.g. bees and wasps) whose colonies have aerial nests that are exposed to the attacks of vertebrate predators, and reduced or removed in most of the ants.

The primitive societies of *Polistes* wasps are defended by such weapons, and these weapons represent a harmful deterrent even for humans (Schmidt *et al*. 1983). The venom, however, is a complex secretion whose study is far from complete and whose properties have not been clarified. Its defensive function seems to be directed to a broad range of enemies, while its communicative role has not been fully investigated. There are various detailed reviews on the composition of Hymenoptera venoms (e.g., Edery *et al*. 1978, Banks & Shipolini 1986, Nakajima 1986, Schmidt 1986, 1990, King & Spangfort 2000); here I focus on the various components of *Polistes* venom and discuss the possible functions of this secretion.

The venom glands

The venomous apparatus of Vespidae, similar to that of other Aculeate Hymenoptera, is extremely complex and formed by various anatomical parts. It is not my intention to discuss this further as there are already extensive reviews on this topic (for example Spradbery 1973, Hermann & Blum 1981, and references therein). Indeed, a detailed description of the venom apparatus of *Polistes annularis* can be found in Hunt and Hermann (1970).

Venom is the secretion that comes out of the tip of the sting and originates (perhaps almost entirely) from the venom reservoir. In wasps

and ants, an organ that produces this secretion includes two tubular glands, which are external to the reservoir, and one gland that is situated inside the reservoir. The last, which is called the convoluted gland, is formed by the glandular epithelia of the tubular glands which infolds like a glove finger inside the reservoir (Schoeter & Billen 1995: fig. 1). A recent ultramorphological study by Britto and Caetano (2005) shows differences in the structure and secretory activity of the two types of glands in *Polistes versicolor*: the tubular glands' cells indicate only a hystochemical positive reaction for proteins, while those of the convoluted gland present a positive reaction for proteins, and a neutral one for glycoconiugates and lipids. These authors suggest that the compounds secreted by the tubular glands could be modified, once in the reservoir, by secretions of the convoluted gland. Powerful muscles which coat the reservoir squeeze the venom, which spurts through a fissure in the internal edge of one of the two sting lancets (for *P. gallicus*, Sledge *et al*. 1999: fig. 2c).

The use of the sting and venom in *Polistes* wasps is not limited to the defence of the colony, and is modulated by various factors (Turillazzi 1984). It is well known that the sting can be used as a weapon in fierce intraspecific and interspecific fights (Pardi 1948). In general, the venom is used in very limited amounts, which suggests that the secretion is costly to produce. In addition, the use of venom against predators is influenced by multiple factors: the parental investment of the colony (Judd 1998, 2000), the temperature, the degree of risk of a given stimulus, the species, etc. If a stimulus induces a wasp to leave the nest and attack (and "stinging may in fact be regarded as an exceptionally powerful anti-vertebrate tactic", Starr [1990]), it is important to note that even when a wasp lands on a possible target it does not necessarily sting it. The venom is released only at the very end of the complete behavioural sequence which includes grasping of the target with legs and mandibles and bending the abdomen (pers. obs.). It is evident that the emission of the secretion is regulated by complex stimuli and depends on an efficient sensorial apparatus of the sting itself (*see* Hermann & Blum [1981], for an extensive bibliography on the argument). Research on this aspect of wasp behaviour is lacking.

The quantity of venom available for a single female *Polistes dominulus* — a medium size and exceptionally common species in the northern hemisphere — is approximately 0.33 μl (E. Francescato pers. comm.). Only a small part of this can be used during each sting. While in some polistine wasps the phenomenon of "venom spraying" is reported (three species in the genus *Parachartergus*; *see* Hermann *et al*. 1993, Jeanne & Keeping 1995), it seems that this defensive option is not practiced by any *Polistes* species. *Polistes* also do not present sting autotomy which is instead present in other polistine wasps belonging to the tribes Epiponini and Ropalidiini (Hermann & Blum 1981, Hermann 1984, Sledge *et al*. 1999).

Chemical composition of *Polistes* venom

There is a significant amount of literature on the chemical composition of venom for other social Hymenoptera. The same cannot be said for the venom of *Polistes*, which consists of a complex mixture of compounds that can be grouped in fractions according to their relative molecular weight (MW): low, medium and high.

The low MW fraction

This comprises various low molecular weight substances and it is probably the least known fraction of the venom from both its chemical and functional aspect. It is known that in social Hymenoptera, including vespids, this fraction contains alarm pheromones (Landolt *et al*. 1998). This has been confirmed for vespine wasps [*Vespula vulgaris* and *V. germanica* (Maschwitz 1964), *Dolichovespula saxonica* (Maschwitz 1984), *V. squamosa* (Landolt & Heath 1987, Landolt *et al*. 1999), *Provespa anomala* (Maschwitz & Hanel 1988), *V. maculifrons* (Landolt *et al*. 1995), *Vespa orientalis* (Ishay *et al*. 1965) and *V. crabro* (Veith *et al*. 1984)] and in polistine wasps [*Polybia occidentalis* (Jeanne 1981, Dani *et al*. 2000), *Polybioides raphigastra* (Sledge *et al*. 1999), *Ropalidia flavopicta* (Fortunato *et al*. 2004)]. However, at present, only a few active

compounds with confirmed alarm pheromone function have been reported [N-(3-methylbutyl) acetamide in *Vespula squamosa* and *V. maculifrons* (Heath & Landolt 1988, Landolt *et al*. 1995), 2-methyl-3buten-2-ol in *Vespa crabro* (Veith *et al*. 1984), and the spiroacetal (E,E)-2,8-dimethyl-1,7-dioxaspirol[5.5]undecane in *Polybia occidentalis* (Dani *et al*. 2000)]. Alternatively, experiments conducted by Keeping (1995) and by London and Jeanne (1996) on the independent founding polistine wasps *Belonogaster petiolata* and *Mischocyttarus immarginatus* excluded the possibility of chemical alarm communication in these species.

For various species of Stenogastrinae, the most primitive of all social wasps (Carpenter 1982), venom volatiles have been reported but behavioural experiments failed to demonstrate any pheromonal function of the secretion (Dani *et al*. 1998). After inconclusive experiments by Freisling (1942) on the existence of pheromonal mediation of alarm in the European *Polistes nimphus* and *P. dominulus*, Maschwitz (1964) excluded the possibility that *P. dubius* (= *P. biglumis*) (subgenus *Polistes s.s.*) had alarm pheromones; this has long been reported as conclusive evidence that *Polistes* cannot provoke alarm by means of chemical communication. However, Jeanne (1982) observed that in the neotropical *P. canadensis*, crushed venom reservoirs elicited alarm in conspecifics and Post *et al*. (1984) described attraction and attack behaviour in response to venom secretions in colonies of the North American *P. fuscatus* and *P. exclamans* (subgenus *Aphanilopterus*). Recently similar experiments were conducted on three species of European *Polistes*. These experiments demonstrated the existence of alarm pheromones in the volatile fraction of the venom of *P. dominulus* at least in the post emergence phase (Bruschini *et al*. 2006a). However, at present all studies on *Polistes* have failed to determine if the alarm response is elicited by the emission of venom by signalling wasps or by the venom released during a sting. Interestingly, Post and Jeanne (1983, 1984, 1985) described a potential sexual action, as male attractant, of the volatile component of the venom of female *P. fuscatus* and *P. exclamans*.

Chemically, the volatile fraction of the venom of *Polistes* was almost completely unknown. Recently we performed gas chromatographic-mass spectrometric analysis of this fraction on four species of European *Polistes* (*P. dominulus*, *P. gallicus*, *P. nimphus* and the social parasite *P. sulcifer*) and on the South Asian *P. olivaceus*. The different species possess a wide range of compounds, and the mixture of which is particular for any species (Table 1) (Bruschini *et al*. 2006c, 2006d), which means that they can be used as systematic characters (Bruschini *et al*. 2006d). The most common compounds are spiroacethals, amides and compounds of acetic and propionic acid. One of the compounds consistently present is N-(3-methylbutyl)acetamide, which is an alarm pheromone in *Vespula squamosa* and *V. maculifrons* (Heath & Landolt 1988, Landolt *et al*. 1995). The exact pheromone for *Polistes* is not yet known, and it may be that various compounds function as an alarm mixture.

Communication of alarm in *Polistes* also relies on vibrational and visual signals (Turillazzi 1984, Starr 1990). A colony of paper wasps with an un-enveloped nest and relatively small size such as those of *Polistes*, would probably gain advantages by substituting a specific alarm pheromone to a vibrational or visual signal only when the colony becomes very large. However, compounds within the venom mixture could have acquired an alarm function in the evolution from simpler to more complex societies. As such, *Polistes* could represent an intermediate step in this evolutionary process.

The unsheathing of the sting and the consequent emission of very small quantities (puffs) of venom volatiles could represent a good system of chemical signalling, and small variations in chemical composition of the mixture could contain information about the signalling individual. I suggest that this aspect deserves investigation both in *Polistes* and other aculeate Hymenoptera.

Other low MW substances

The low molecular weight fraction of Vespid venom includes biologically active amines. In *Polistes*, serotonine, histamine, tyramine and dopamine are reported (Nakajima 1985). In *P. humilis* (as well as in *Ropalidia revolutionalis*)

Table 1. Presence of volatile compounds in the venom of *P. dominulus* (*P. d.*), *P. gallicus* (*P. g.*), *P. nimphus* (*P. n.*), *P. sulcifer* (*P. s.*) and *P. olivaceus* (*P. o.*). (Modified from Bruschini *et al.* 2006d).

n[a]	Compound	MW	*P. d.* *n* = 10	*P. g.* *n* = 11	*P. n.* *n* = 11	*P. s.* *n* = 10	*P. o.* *n* = 7
1	7-Methyl-1,6-dioxaspiro[4.5]decane	156					+
2	Unidentified		+				+
3	2-Methyl-1,6-dioxaspiro[4.5]decane	156					+
4	2-Methyl-1,6-dioxaspiro[4.5]decane	156					+
5	(*E*)-4,8-Dimethyl-1,3,7-nonatriene	150	+			+	+
6	Unidentified						+
7	6-Methyl-5-hepten-2-yl acetate	170	+	+	+	+	+
8	(*E*,*E*)-2,8-Dimethyl-1,7-dioxaspiro[5.5]un decane	184	+	+			+
9	N-(3-Methylbutyl)acetamide	129	+	+	+	+	+
10	2-Ethyl-7-methyl-1,6-dioxaspiro[4.5]decane isomer A	184	+	+			+
11	2-Ethyl-7-methyl-1,6-dioxaspiro[4.5]decane isomer B	184	+	+			+
12	2-Methyl-7-ethyl-1,6-dioxaspiro[4.5]decane	184	+	+			
13	Unidentified		+				+
14	2,8-Dimethyl-1,7-dioxaspiro[5.5]undecane	184	+				+
15	N-(3-Methylbutyl)propanamide	143	+	+		+	
16	Unidentified		+	+	+		+
17	2-Nonanyl acetate	186	+	+	+	+	+
18	Unidentified						+
19	Undecen-2-ol	170	+	+			
20	2-Undecanone	170	+	+	+		
21	2-Undecanol	172	+	+	+	+	
22	2-Nonanyl propanoate	200	+	+			
23	2-*n*Propyl-8-methyl-1,7-dioxaspiro[5.5]undecane	212		+			+
24	Unidentified			+			+
25	Unidentified						+
26	Spiroacetal	198					+
27	Unidentified						+
28	Unidentified		+				
29	Unidentified		+				
30	Unidentified		+				+
31	Unidentified		+		+		
32	Unidentified		+		+	+	+
33	Unidentified		+				+
34	2-Undecenyl acetate	212	+	+	+	+	+
35	2-Undecanyl acetate	214	+	+	+	+	+
36	6,10-Dimethyl-(*E*)-5,9-undecadien-2-one (Geranyl acetone)	194	+	+	+	+	+
37	2-Undecenyl propanoate	226		+			
38	2-Undecanyl propanoate	228		+			
39	Unidentified		+				+
40	Unidentified		+				+
41	6,10-Dimethyl-(*Z*)-5,9-undecadien-2-yl acetate ((*Z*)-5-Tangerinol)	238	+		+	+	+
42	6,10-Dimethyl-(*E*)-5,9-undecadien-2-yl acetate ((*E*)-5-Tangerinol)	238	+	+	+	+	+
43	2-Tridecenyl acetate isomer A	240	+	+	+	+	+
44	2-Tridecenyl acetate isomer B	240	+	+	+	+	+
45	2-Tridecanyl acetate	242	+	+	+	+	+
46	6,10-Dimethyl-(*E*)-5,9-undecadien-2-yl propanoate	252		+			
47	2-Tridecenyl propanoate isomer A	254		+			
48	2-Tridecenyl propanoate isomer B	254		+			
49	2-Tridecanyl propanoate	256		+			
50	Unidentified		+		+	+	+
51	Unidentified		+		+	+	
52	2-Pentadecanyl acetate	270	+	+	+	+	+
53	3,7,11-Trimethyl-(*E*)6,(*E*)10-dodecatrien-2-yl acetate ((E,E)-Farnesyl acetate)	264	+	+	+	+	
54	2-Pentadecanyl propanoate	284		+			

[a] *n* = number corresponding specimen examined.

Owen (1979) reported the presence of 5-hydroxytryptoamine. The pharmacological action of these substances is unknown, but Nakajima (1985) considered them the major pain producing components in the venom. Eno (1997) observed that in the sting of an African species of *Polistes* (which the author identifies as *P. fuscatus* even though this species is not reported in Africa; Carpenter [1996]) histamine and serotonine, combined with kinins and lipogenase derivatives, are probably involved in venom-induced oedema. Nothing is known about the possible correlation between the presence and quantity of these substances and caste or age in any Vespidae — even though the toxicity of venom increases with the age in *Vespa orientalis* (Edery *et al*. 1978).

Peptidic fraction

The medium molecular weight components (roughly from 900 Da to 3000 Da) of the venom can be defined as the peptidic fraction, as it is mainly composed by short aminoacid chain peptides. This fraction is also largely unknown in *Polistes*, as it is for the venom of other Aculeates.

Kinins

Kinins are polypeptides of 9–18 amino-acid residues containing a bradykinin-like sequence (bradykinin is a 9-amino-acid substance discovered in mammalian blood serum which is active on a variety of smooth muscles). A similar substance was discovered in the venom of *Vespula vulgaris* and was called wasp kinin (Jaques & Schachter 1954, Schachter & Thain 1954); other kinins were described in the venom of other social and solitary wasps. In general, kinins have been found in venoms of species belonging to various families of Vespoidea (including Vespidae and Formicidae but excluding the Pompilidae) but not in the Apoidea. *Polistes* were the very first genus in which the primary structure of a kinin was described. An octadecapeptide named Polisteskinin 3 was found in the venom mixture of three North American species (*P. fuscatus*, *P. exclamans* and *P. annularis* (Udenfriend *et al*. 1967). Watanabe *et al*. (1976) reported the Polisteskinin R in the venom of the Japanese species *P. rothneyi iwatai*. In *P. jadwigae* Nakajima *et al*. (1984) described two forms of kinins with 11 residues, differing for an amino acid in the second position, which they called Polisteskinin J. The same authors described one more molecule (Polisteskinin C) in the venom of *P. chinensis* differing from the previous ones in the first two amino acids (Nakajima *et al*. 1984). More recently, Uzbekistani researchers isolated and sequenced six vasoactive peptides from the venom of *Polistes gallicus*, four of which are structural analogues of bradykinins (L'vov *et al*. 1990, Mukhamedov *et al*. 1991) (*see* Table 1). Both Nakajima (1986) and Piek (1990) observed that components of the venom of both solitary and social wasps (and ants) may have different functions: contributing to permanent paralysis of the prey and to the defence of the colony against vertebrate predators. In fact, kinins are the main pain producers of the venom against vertebrates and represent a repellent against colony threats; at the same time they may be effective against conspecifics or congenerics in the case of aggressive interactions from usurpers or social parasites. On this point, Schmidt *et al*. (1984) found that vespid social parasites did not develop a special toxic venom against their hosts.

Mastoparans

Mastoparans are tetradecapeptides that usually contain between 7 and 10 hydrophobic amino acid residues and between 2 and 4 lysine residues in their primary sequence. Peptides included in this class have been found in the venom of various social and solitary vespids (Mendes *et al*. 2005). The first described was that of *Vespa xanthoptera* (Hirai *et al*. 1979), which was named Mastoparan X. The first Mastoparan described for a *Polistes* wasp was that of *P. jadwigae* from Japan (Hirai *et al*. 1980) (*Polistes* mastoparan). It is known that these substances can induce haemolysis in animal cells (Schmidt *et al*. [1983] reported that the 12 000 hemolytic units/mg of dry venom of *P. infuscatus* (subg. *Aphanilopterus*) represent the highest reported for any insect venom) and mast-cell degranulation. It is

also known that these substances can activate venom enzymes (Mendes *et al*. 2005). Recently our group described two eptadecapeptides found in the venom and on the cuticle of *Polistes dominulus* that can be included in the class of Mastoparans (*see* Table 2). The two substances, named Dominulin A and Dominulin B (Turillazzi *et al*. 2006c) present a strong antimicrobial activity against some Gram+ and Gram– bacteria (and a lower but significant activity against *Candida* fungi) and were found on the females' cuticle, in their venom, and on the nest surface. Turillazzi *et al*. (2006c) hypothesized that the dominulins are produced in the venom, but are spread all over the body via cleaning movements, and contribute to form a barrier against microbial pathogens. Antimicrobial activity of venom has been reported for other social insects (including social wasps), but for only *Vespa affinis* was it demonstrated that this was due to a mastoparan peptide (Park *et al*. 1995). The case of *P. dominulus* demonstrates that venom can be used for individual and colony protection against enemies other than vertebrates or invertebrates.

A further function of the dominulins has been reported by Turillazzi *et al*. (2006b) who found that these substances are within the suite of chemical cues used by future *P. dominulus* foundresses to assess the suitability of places to hibernate. In fact, large quantities of these peptides are deposited year after year by hibernating wasps in habitually used hibernacula.

Similar to the volatile fraction, the peptidic fraction of the venom is species-specific. In a recent study, Turillazzi *et al*. (2006a) found interesting differences in the spectral profiles in the MW range from 900 to 3000 Da of the venoms of three European (*P. dominulus*, *P. gallicus* and *P. nimphus*) and one North American species (*P. exclamans*). These differences may be important in the development of a reliable method for quality control of venoms used in immunotherapy.

Proteic fraction

The high MW fraction of Hymenoptera venom is composed by various proteins, some of which have documented enzymatic activity. This is probably the most studied fraction in Aculeates, as some proteins can be powerful antigens and cause strong allergic reactions in humans and animals. Allergic reactions can have local or systemic characters; the systemic ones are by

Table 2. Amino acid sequences of kinins and mastoparans peptides reported in the venoms of various species of *Polistes*. In the kinins table the sequence of Bradikinin is also reported for comparison. References: (1) Udenfriend *et al*. 1967, (2) Watanabe *et al*. (1976), (3) Nakajima *et al*. (1984), (4) Mukhamedov *et al*. (1991), (5) Hirai *et al*. (1980), (6) Turillazzi *et al*. 2006c.

KININS		
R P P G F S P F R	Bradikinin	
T N K K K L R G R P P G F S P F R	Polisteskinin 3	N. American *Polistes* (1)
A R R P P G F T P F R	Polisteskinin R	*P. rothney* (2)
A R R P P G F T P F R	Polisteskinin J	*P. jadwigae* (3)
A T R P P G F S P F R		— ” —
S K R P P G F S P F R	Polisteskinin C	*P. chinensis* (3)
I K A G G I V K K K L	Peptide I	*P. gallicus* (4)
F K V P K K G V F T S P L	Peptide V	— ” —
L A I P F C F G R P P G F S P F R	Peptide II	— ” —
F K L V K R P P G F S P F R	Peptide III	— ” —
I R P P G F S P F R V	Peptide IV	— ” —
I R P V G F S P F R T S	Peptide VI	— ” —
MASTOPARANS		
V D W K K I G Q H I K S V L	Polistesmastoparan	*P. jadwigae* (5)
I N W K K I A E V G G K I L S S L	Dominulin A	*P. dominulus* (6)
I N W K K I A E I G K Q V L S A L	Dominulin B	— ” —

far more serious and range from a generalized urticaria to protracted anaphylaxis, which can be fatal (Golden 2005). Hoffman (2004) provided a review of principal studies of Hymenoptera venom allergens but, at the same time, points out how much of the research performed on different allergenic components were altered by substances derived from the venom sac extract or from the digestive tract. Vespid venom contains three to five important protein allergens, but several trace proteins, which could be minor allergens, are also present (Hoffman 2004). Differences in the primary structure of these proteins vary according to genera and species, and biochemical studies increasingly contain immunological research.

In *Polistes* wasps, the principal proteins are Antigen 5, Phospholipases, Hyaluronidases and Proteases (Hoffman 2004), and at least some of these are glycoproteins (Hemmer *et al*. 2001). Proteins belonging to the last three classes are important enzymes; Antigen 5 is likely an invertebrate neurotoxin (Hoffman 2004) that was named after the fraction number of the chromatographic peak found by King *et al*. (1978) in the analysis of the venom of *Dolichovespula maculata* and other vespines. A large number of patients who are allergic to vespid venom may exhibit reactions to more than one species, and this may be caused by cross reactivity to one or more allergens with the production of IgE recognizing common epitopes. Usually venom allergens with less than 70% of sequence identity do not induce high cross reactivity. On this basis, the venom of *Vespa*, *Vespula* and *Dolichovespula* species are much more similar to each other in amino acid sequence than to the venoms of *Polistes* (Reismann *et al*. 1982, Grant *et al*. 1983, Schmidt 1986, Sanchez *et al* 1995, King & Spangfort 2000, Pantera *et al*. 2003, Hoffman 2004).

Regarding *Polistes*, differences in the allergy reactions to venom of European (*P. dominulus*) and North American *Polistes* were first noted by Hoffman *et al*. (1990). Studies on specific IgE in large group of sera from patients sensitized to the European species *P. dominulus* and *P. gallicus* tested with European and North American *Polistes* venoms (*P. exclamans*, *P. fuscatus*, *P. metricus*, *P. annularis* and *P. aphacus*) indicated a high percentage (97.8%) of positive cases of cross reactivity. Lower percentages of cross reactivity were observed with North American *Polistes* venom, with significant differences in the RAST (radioallergosorbent test) values (Sanchez *et al*. 1995, Severino *et al*. 1998, Severino *et al*. 2006). These results show that a specific allergenic difference exists between North American and European species.

The phospholipases of *Polistes* are of the A1 type and are different from the phospholipase A2 of honey bee venom. As Schmidt (1982) observed, phospholipases are the only known toxic enzymes in venom that are present in the venom of most organisms and act strongly as antigens. The only species of *Polistes* in which one of these proteins has been completely sequenced are the North American *P. annularis* (Swiss Pro Databank n. Q9U6W0) and the European *P. dominulus* (Genbank n. AY566645; Moawad *et al*. 2005). For the European *P. gallicus*, only the sequence of the first 43 residues has been determined (Pantera *et al*. 2003). The mass weight of the proteins is similar in the three species (*P. annularis* 33483 Da; *P. dominulus* 33502.3 Da; *P. gallicus* 33475 Da). The sequence identity was 75.4% between *P. annularis* and *P. dominulus* (Moawad *et al*. 2005) and 62% in the 1–43 stretch between *P. annularis* and *P. gallicus* (Pantera *et al*. 2003).

The only *Polistes* hyaluronidase that has been sequenced is that of *P. annularis* (Swiss Pro Data Bank n. Q9U6V9) with a mass weight of 43019 Da.). *P. gallicus* hyaluronidase has a MW of 43173 Da, determined by MALDI/TOF mass spectrometry. Vespid and honeybee hyaluronidase seems quite similar, and the cross reactivity found in patients allergic to the venom of apids and vespids is probably due to the presence of IgE antibodies against this enzyme (Hoffman 2004). These enzymes hydrolyze the mucopolysaccharid polymers that constitute the bulk of animal connective tissue and contribute to "open the passages" through the skin for other venom components.

Recently, proteins recognized as serine protease enzymes have been reported in the venom of honeybees (Schmidt *et al*. 2002) and bumblebees (Hoffman *et al*. 2001), and also in the venom of *Polistes*, which represent the

only vespids in which these proteins have been found (Hoffman *et al*. 1998, McNairy *et al*. 2000, Pantera *et al*. 2003, Winningham *et al*. 2004). The protease of *Polistes dominulus* has been completely sequenced (mRNA sequence deposited in GenBank with accession number AY285998) (Winningham *et al*. 2004), and the proteases of *P. exclamans* (McNairy *et al*. 2000) and *P. gallicus* have been partially sequenced; in *P. gallicus* the MW determined by MALDI/TOF mass spectrometry is 33669 Da but only the 1–16 N-terminal sequence has been identified (Pantera *et al*. 2003). Immunologically, the proteases of the venom of honeybee, bumblebee, and paper wasps do not show important cross reactivity (Winningham *et al*. 2004). However, these authors found that enzymes from the venom of a North American *Polistes* (*P. exclamans*) are highly cross reactive with the venom of the European species *P. dominulus* and *P. gallicus*. At the same time, Pantera *et al*. (2003) found that proteases are weak sensitizers in N.A. *Polistes* venom, while they are significant allergens in European species.

Of all venom proteins, Antigen 5 seems the most powerful antigen in vespids; this is also a non enzymatic protein, the function of which is unknown. In *Polistes*, Antigen 5 of various North American species [*P. annularis* and *P. exclamans* (Lu *et al*. 1993) and *P. fuscatus* (Hoffman 1993)] and of the European *P. dominulus* (Swiss-Prot Data Base n. P81656) and *P. gallicus* (Pantera *et al*. 2003) have been completely sequenced. When the Antigen 5 of *P. annularis* (Swiss Pro Data Bank n. Q05109, MW = 23293 Da) and *P. gallicus* (MW = 23135 Da, 206 residues) were compared for the amino acid sequence and for the tertiary structure, a number of differences were detected in the superficial loops which represent putative species-specific epitopes (*see* Pantera *et al*. 2003: fig. 9). These data suggest that this protein is the major cause of the different responses to the venoms of the American and European species.

After the classic work of Schmidt (1986), Golden (2005) recently produced an interesting review of the art of venom immunotherapy. Venom immunotherapy is recommended for patients at high risk for sting reactions, and after 5 years of treatment the chance of systemic (but mild) reaction is reduced to 10% (Golden 2005). However, treatment should be done with the venoms of all the species resulting in a positive skin test or RAST; this explains the importance of the identification of the correct species to be used for the immunotherapy. Recently, the application of molecular biology techniques has led to research on therapies with recombinant proteins similar to those found in venom extracts (King *et al*. 2001, Winkler *et al*. 2003).

The differences in venom antigen sequence between various genera and species helps explain differences in cross reactivity to different venoms by allergic patients. This raises the possibility that these biochemical characters — and perhaps even the pharmacological reactions to venoms — may be useful in phylogenetic studies. Examples of this form of study are well known starting with Piek (1987), who considered the structure of wasp kinin. Zalat (1997) produced a phylogenetic analysis of Vespid wasps based on the biochemical composition of their venom: species of *Polistes*, *Vespa* and *Dolichovespula* were compared for differences in the electrophoretic bands obtained from biochemical analysis of their venoms. More recently, Pantera *et al*. (2003) attempted to reconstruct a phylogenetic tree of the Vespidae by the alignment of the available Antigen 5 sequences for 5 species of *Polistes*, 2 of *Dolichovespula*, 2 of *Vespa* and 7 of *Vespula*. Their phylogenetic tree (Pantera *et al*. 2003: fig. 8) fits well with the trees developed on morphological and behavioural characters (Carpenter 1991). *Polistes* in particular are arranged according the different subgenera with the European *P. gallicus* and *P. dominulus* belonging to the subgenus *Polistes s.s*. and the North American *P. exclamans*, *P. annularis* and *P. fuscatus* to the subgenus *Aphanilopterus* (Carpenter 1996).

Conclusions

The evolution of exocrine apparatuses in social insects distinguish them from solitary insects (Billen & Morgan 1998). The evolution of social vespids from solitary ancestors resulted in significant changes in the behavioural repertoires and in exocrine glands anatomy and physiology and of chemical products (*see* Hermann &

Blum 1981). Venom represents the most complex secretion produced by aculeate Hymenoptera. This secretion, originated as a tool for the capture and storage of prey in solitary species, became a weapon for defending the colony in social species. As such, the study of venom represents an additional character from which to study social evolution. *Polistes* wasps represent a suitable study subject for this argument, as they are an intermediate step between solitary and highly eusocial wasps. In this review I described the current status of research on *Polistes* venom. As in other social Hymenoptera, I can confirm that the main functions of the venom deal with colony (and individual) defence and with chemical communication. Venom has various components that can be useful for defence against different types of colony vertebrate and invertebrate enemies.

It is highly likely that particular compounds of the secretion are also used as antimicrobial agents for the defence of the colony from pathogens. The defensive role of venom extends to alarm communication, which is now demonstrated in species of two subgenera. The identification of the actual alarm pheromone of *Polistes* will represent a challenge for future researchers, as the particular evolutionary position of this genus, and the contemporary use of other alarm signals, will make the bioassays quite difficult. At the same time, research on the sexual attractiveness of venom for males should be extended to other species and coupled with chemical analyses to clarify the communicative value and the actual compounds involved in the phenomenon.

Why do we need research on venom chemistry? The answer is obvious from a medical and pharmacological point of view: we hope to find new substances with pharmacological activity [for example, wasp mastoparan was recently used as a transduction peptide to transfer large drugs molecules through the cell membranes (*see* Fuchs *et al*. 2005)], while a broad study of the allergens of the various species will represent an important contribution to the development of a more reliable and effective venom immunotherapy for allergic people. Insect sting allergy and venom immunotherapy is considered an excellent model for the study of immune tolerance and anaphilaxis, even if their basic mechanisms remain to be clarified (Golden 2005).

The study of communicative properties of venom may result in interesting discoveries. This secretion, in fact, has a ductile apparatus of distribution, which can inflict painful stings but also distribute regulated emissions of chemical volatiles. For example, the study of variation in venom compounds in different castes and age groups could be important for understanding the roles of individuals in the social organization of colonies. Research on chemical communication may also have applied science value: for example, artificial sex attractants could be used in campaigns to regulate wasp populations. A final reason for implementing research on venom is that the chemical study of this secretion can be useful for taxonomical purposes; this may be true not only for the amino acidic sequence of the big proteic molecules but also for the medium MW fraction and for the "bouquet" of the more volatile compounds.

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