

EDITORIAL

Let's not forget crawling Hymenoptera

We are all aware of flying Hymenoptera that sting and induce hypersensitive reactions. These insects, the wasps (called yellow-jackets in North America), hornets, *Polistes* wasps, honey bees, and a few others, capture one's attention and imagination because they attack by flying directly at and pursuing the victim. People feel vulnerable to this mode of attack as there is no easy protection or escape. But what about venomous crawling Hymenoptera from which escape seems readily possible? Have we overlooked the potential of allergy from stinging ants? Stinging ants far outnumber their flying counterparts in both species numbers and total ecological biomass [1], yet knowledge of their venoms and allergenicity is emerging only slowly. Should we be concerned about ant allergy in our patients?

Although detailed investigations of ant venoms are rare, a few generalities have emerged. Most ant venoms are blends of proteins analogous in activity to those of bees and wasps (Table 1). Virtually all contain phospholipase, or evidence of phospholipase activity, and hyaluronidase [2]. Other enzymes including lipase, esterase, alkaline and acid phosphatases, and proteinases are

present in some ant venoms [3]. Like bees and wasps, many ant venoms contain peptides including known allergens such as kinins [4] and haemolysins [5,6]. A variety of other medically insignificant components such as histamine and other active amines are also found in ant venoms.

The convergence of pharmacological activities among the venoms of wasps, bees, and ants leads to the intuitive conclusion that these venoms are biochemically and immunologically similar. Here Mother Nature takes us by surprise—whatever similarity has been observed is based on taxonomic relatedness irrespective of pharmacological or biochemical activity. Hymenoptera venoms represent one of the grandest examples of evolutionary convergence. Ant, wasp, and bee venoms all have the primary function of repelling large predators [7] and there are basically two ways to accomplish that: produce pain and cause physiological damage. Phospholipases, hyaluronidases, and algogenic and toxic peptides are the convergent means of producing pain and damage (snake venoms also contain components with these activities).

Clinical investigation of ant venom hypersensitivity is

Table 1. Protein composition of Hymenoptera venoms*

	Honey bees	Vespid wasps	Fire ants	Harv ants	Bull ants	Other ants**	References
<i>Enzymes</i>							
Phospholipase A ₁	0	+	***	***	***	***	[3,14,19,22,23]
A ₂	10–12%	0					[24]
B	1%	+	+	+			[3,14,19,24]
Hyaluronidase	1–2%	+	0	+	+	+	[2,3,24,26]
Acid phosphatase	1%	0	+	+		+	[3,24,25,26]
Alkaline phosphatase	+	0		+		+	[3,25]
Lipase	0	+		+		+	[unpublished,3]
Esterase	+	+		+		0	[3,25]
Protease	0	***	0	0		+	[3]
<i>Peptides</i>							
Haemolysins	melittin 40%	+		barbatolysin	+	+	[5,6,24]
Mastolytic peptides	MCD-peptide 2%	mastoparans					[24,27]
Neurotoxins	apamin 3%	+		+		+	[22,24,27,28,unpublished]
Antigen 5	0	+	+		?		[15]
Kinins	0	+		+	0	+	[4,23,27]
Group specific allergens	+	+	+		+		[11,12,15]

* Symbols: +. Present. 0. Absent. No symbol. Not investigated.

** Detected in at least one species; not necessarily in all species.

*** Specificity unknown.

in its infancy compared with that for bee and wasp venoms. The reasons for this are varied: ants are difficult to identify, ant venoms are difficult to obtain, most stinging ants live in the tropics, and stinging encounters with ants are usually easy to avoid. Two locations in the world where conditions are ideal for clinical investigations of ant venom hypersensitivity are the southeastern United States and Australia. In both of these areas large populations of pugnacious stinging ants present an omnipresent risk to the unwary person. The fire ant *Solenopsis invicta* inhabits much of southeastern USA where in recent years it has become a major medical problem. It is the main cause of insect sting hypersensitivity in the region [8], and is responsible for at least 32 human deaths [9]. Street *et al.* [10] in this issue of the journal detail immediate allergic reactions to Australian bullants of the genus *Myrmecia* and probe the question of crossreactivity among the venoms of six species of these ants. This question is particularly important for gaining an understanding of venom hypersensitivity in general and its treatment in specific. Early investigators noted that patients often presented a mixed picture vis-à-vis crossreactivity among the venoms of different stinging species [11,12]. Some patients exhibited positive skin or RAST tests only to one species, while others were positive to a variety of species, including species that did not live in the area. Explanations were that the venoms either contained crossreactive proteins, or that the patients had developed hypersensitivity to two or more species through independent exposures. Because patient memories and insect identification capabilities were not adequate, allergists often could not distinguish between the two explanations. As a consequence the conservative use for immunotherapy of all venoms eliciting a positive skin test evolved.

Recently, thanks to the use of modern immunological, biochemical and molecular techniques, a much better picture of the relationship between true crossreactivity and multiple reactivity is emerging. Biochemical isolations and characterizations have yielded complete sequences and structures of many venom allergens [13–16]. Through the use of molecular techniques and cDNA libraries several more venom allergens have been identified and sequenced, including the major allergen in the venom of *Myrmecia pilosula* [17]. The sequences of various allergens can now be compared by computer matching with other known molecules listed in sequence banks. This has recently been done for 15 different antigen 5 molecules originating from the venoms of an assortment of wasps, hornets, *Polistes* wasps, and the fire ant [15]. The findings from this analysis revealed the following: the antigen 5 molecules from wasps in the *Vespula vulgaris* species group were over 93% identical;

the species in this group shared antigen 5 similarities only in the low 70% range with two other species in the *Vespula* (those two shared 80% crossidentity); two distinctive species in the sister genus *Dolichovespula* possessed antigen 5 molecules about 77% identical and which shared identities with the *vulgaris* group in the low 60% range. All of these species in the subfamily Vespinae shared only 57–63% identity with the venoms of three *Polistes* wasps in the sub-family Polistinae. Ants are in the family Formicidae, a family somewhat related to the wasp family Vespidae [18], but nevertheless are separated by a much greater taxonomic distance from any of the wasps than the wasps are among themselves. The antigen 5 from the fire ant exhibited a nearly identical low to mid 40% similarity to each of the wasp molecules. The degree of crossreactivity of patient sera with these various venoms often mirrors the identity relationships of the antigen 5 molecules. The sera have virtual total cross-reactivity to all *vulgaris* wasp venoms, partial cross-reactivity among the wasp genera, and lack detectable crossreactivity between fire ant and any wasp venom [15]. There is some crossreactivity of fire ant and vespid wasp venoms for some sera; but this is a result of cross-reactivities between phospholipases, molecules that appear fundamentally more conservative in structure than antigen 5. The story of the phospholipases is in its genesis [19].

Street *et al.* [10] discovered two patterns among sera from 93 ant venom allergic patients. The majority of sera were reactive only to the venom of *Myrmecia pilosula*, but one-third showed multiple reactivities, often to all six *Myrmecia* (including *pilosula*) venoms tested. Thus, one can conclude that among the six examined ant species, some venom antigens are unique to *pilosula*, and others are similar enough to be crossreactive among species. At the molecular level, of the six species only *M. pilosula* lacked an antigen of molecular weight of 25 kDa. Could it be that the 25 kDa peptide antigens are actually antigen 5 molecules (antigen 5 molecules have a MW of 24–25 kDa) which are similar and crossreactive among species, and that *M. pilosula* in lacking that peptide is distinct, but has other unique antigens? It will be most informative to learn the sequence of the 25 kDa allergen and to compare its structure to known antigen 5 molecules.

The ant subfamily Myrmeciinae presents a particularly rich opportunity to test the strength of taxonomic prediction of the outcomes of allergy and clinical immunology. At the moment the subfamily contains at least 89 species, which are all lumped into the single enormous genus *Myrmecia* [20]. The genus is divided into nine 'species-groups' [21] which in actuality might have differences among themselves as large as between some

genera of vespid wasps. Indeed, *M. pilosula* differs from many of the other ants in the genus in terms of behaviour, size, and even common name. It is also placed in a distant branch from the other five closely related species in the study of Street *et al.* [10]. We await further investigations of crossreactivity among the *Myrmecia* to see if the venoms in that genus behave more like the yellow-jacket genus *Vespula* or more like the entire subfamily Vespinae.

Stinging ants are an unrecognized source of hypersensitivity in many parts of the world. Numerous species of stinging ants abound throughout the tropics [1] where the local human population is well aware of them. Stinging species including *Myrmica* in Northern Europe, the UK, and Northern North America, harvester ants (*Pogonomyrmex*) of the New World, and the little fire ant (*Wasmannia*), a tropical tramp species, are likely responsible for undiagnosed cases of hypersensitivity. Unfortunately, until we learn more about stinging ant venoms, and these venoms become commercially available, we might be missing an opportunity to assist many patients in need.

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