Journal of Insect Physiology 59 (2013) 186–204

Contents lists available at SciVerse ScienceDirect

Journal of Insect Physiology

journal homepage: www.elsevier.com/locate/jinsphys

"It stings a bit but it cleans well": Venoms of Hymenoptera and their antimicrobial potential

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ARTICLE INFO

Article history:
Available online 13 October 2012

Keywords:
Hymenoptera
Venom
Insect immunity
Microorganisms
Antimicrobial peptides

ABSTRACT

Venoms from Hymenoptera display a wide range of functions and biological roles. These notably include manipulation of the host, capture of prey and defense against competitors and predators thanks to endocrine and immune systems disruptors, neurotoxic, cytolytic and pain-inducing venom components. Recent works indicate that many hymenopteran species, whatever their life style, have also evolved a venom with properties which enable it to regulate microbial infections, both in stinging and stung animals. In contrast to biting insects and their salivary glands, stinging Hymenoptera seem to constitute an under-exploited ecological niche for agents of vector-borne disease. Few parasitic or mutualistic microorganisms have been reported to be hosted by venom-producing organs or to be transmitted to stung animals. This may result from the presence of potent antimicrobial molecules in venoms, histological features of venom apparatuses and selective effects of venoms on immune defenses of targeted organisms. The present paper reviews for the first time the venom antimicrobial potential of solitary and social Hymenoptera in molecular, ecological, and evolutionary perspectives.

1. Introduction

I will long remember my very first entomological experience, 30 years ago. While I was rolling in a park's lush grass with one of my young colleagues (I do not have much hope that it is going to happen again), I suddenly felt my left hand painfully coming into contact with an unexpected encounter. By its furious drone and color, identifying it as a honeybee forager was an easy thing to do, even for the junior entomologists we were. The 2 mm long autotomized aculeus was deeply rooted in the palm of my hand and 50–70 μg of venom proteins (Hoffman and Jacobson, 1984) quickly diffused intradermally between my life and fate lines. Retrospectively, it would be tempting to see here a sort of sign of what was to follow in my professional career, mainly dedicated to the study of Hymenoptera venoms. But the rationale also suggests that, within a few centimeters, another part of my anatomy less open to interpretation could just as easily have been stung!

Strikingly, my friend's mother appeared at least as panicked as the dying bee. Everything in her reaction seemed to mean that I was also sentenced to a nearly inevitable death. She was sharing then a widespread belief considering Hymenoptera stings as extremely dangerous. However, fatal reactions upon insect stings are rare: while virtually the whole adult population living under a moderate climate has been stung at least once (Antonicelli et al., 2002), only 0.03–0.48 fatalities per 1,000,000 inhabitants occur each year (Mosbech, 1983; Biló et al., 2005; Langley, 2005; Liew et al., 2009). In the United States for instance, the probability of dying following contact with hornets, wasps or bees would be in the same order of magnitude (odds of 1 in 71,623) as being struck dead by lightning (1 in 84,079) or legally executed (1 in 96,691) (National Safety Council, 2011). Although the true prevalence of mortality induced by insect stings may be underestimated and is unfortunately still too high, it appears thus quite infrequent. In most cases, it mainly results from the consequences of a severe respiratory blockage or systemic anaphylactic reaction (Schmidt, 1986a). Death may also be caused by renal failure, vascular or neurological injuries, or even by extreme fright (Schwartz et al., 1986; Fitzgerald and Flood, 2006).

More probably, I could have suffered from a moderate systemic anaphylactic reaction or from a large local reaction. The prevalence of systemic anaphylactic sting reactions in a general population is between 0.4% and 7.5% while the prevalence of large local reactions ranges from 2.4% to 26.4% depending of the investigated populations (Reisman, 1994; Biló et al., 2005). In beekeepers daily exposed to bee stings, the prevalence of large local reactions is as high as 38% (de la Torre-Morin et al., 1995; Annila et al., 1996). Fortunately it was not my case. After half an hour it became evident that I would neither succumb immediately nor suffer serious

Abbreviations: AMP, antimicrobial peptide; Ip, intraperitoneal injections; Iv, intravenous injections; PDV, polydnavirus; PLA2, phospholipase A2; VLP, virus-like particle.

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http://dx.doi.org/10.1016/j.jinsphys.2012.10.005

symptoms except a local pain and a limited inflammation. My young colleague seemed to be more sorely affected in being consequently deprived from an extra-part of cake at snack time. His mother, who had become a bit calmer and who was eager to do something for me, decided then to fetch a bottle of disinfectant. "It stings a bit but it cleans well" she said, and effectively, it stung. But was it absolutely necessary? Have we ever heard that a sting inflicted by a Hymenoptera can infect immediately? If it is not the case, what is this due to and what is the adaptive significance of the antimicrobial properties of Hymenopteran venoms? This review focuses on these unresolved issues, emphasizing a neglected functional dimension of venoms of solitary and social Hymenoptera, as effectors of antimicrobial immunity.

2. Biological roles of venoms of the Hymenoptera: a global overview

Venoms of apocritan Hymenoptera (gathering the Terebrantia and Aculeata suborders) fulfill a wide variety of functions ranging from manipulation of the physiology of stung hosts, in solitary parasitoid species, to defense against competitors in social Hymenoptera.

Parasitoid wasps of the Terebrantia suborder use their stinging organ (terebra) to deposit their eggs inside or outside the body cavity of invertebrate hosts (Beckage and Gelman, 2004; Pennacchio and Strand, 2006). Venom secretions produced by ectoparasitoid wasp females often serve to transiently or permanently paralyze the host before oviposition (Askew and Shaw, 1986). In Eulophidae and Pteromalidae families, venom can also arrest or slow growth and development of envenomated hosts (Coudron and Brandt, 1996; Coudron et al., 2000; Weaver et al., 2001; Deyrup et al., 2006) or impair their immune responses (Rivers et al., 1999, 2002; Abt and Rivers, 2007). Venoms from endoparasitoids can induce host castration (Digilio et al., 2000), developmental arrest (Digilio et al., 1998), endocrine alterations (Zhu et al., 2009) and paralysis (Moreau et al., 2002; Mabiala-Moundoungou et al., 2009). They can also directly affect cellular and humoral immune responses in the host (Parkinson and Weaver, 1999; Richards and Parkinson, 2000; Cai et al., 2004; Zhang et al., 2005). In several braconid species, venom acts synergistically with other factors of virulence produced by parasitoid females, such as ovarian proteins and polydnaviruses (PDVs), or teratocytes issued from the developing parasitic embryos (Asgari, 2012). Together these factors contribute to manipulation of host immunity, behavior, and reproductive and developmental metabolisms to the benefit of the parasitoid's offspring (Beckage and Gelman, 2004; Moreau et al., 2009a). The venom composition in parasitoids greatly varies upon species, even within the same genus, hence reflecting their important functional diversification during evolution. Recently however, few ancestrally-inherited venom proteins were found to be conserved between distant hymenopteran species (de Graaf et al., 2010; Vincent et al., 2010; Zhu et al., 2010). Gall wasps (e.g. from the Cynipidae family) also inject venom in host plants during oviposition (Bronner, 1985) but little is known on the functions and composition of these secretions (Vårdal, 2006).

Terebrantia and Aculeata share common parasitic ancestral origins (Grimaldi and Engel, 2005). In the latter suborder however, the ancestral ovipositor has been fully modified for injection of venom (aculeus) and no longer serves for the egg-laying function except in primitive families (e.g. Drynidae and Chrysididae) (Piek, 1986). Many solitary aculeate predators (e.g. Eumenidae, Scoliidae and famous spider wasps from the Pompilidae family) use their venom to induce in their prey a non-lethal paralysis which can last from a few minutes to several weeks if the prey is not consumed (Piek and Spanjer, 1986; Hisada et al., 2005; Yamamoto et al., 2007; Baek and Lee, 2010). The venoms of some aculeate parasitoids (e.g. in sphecid species) induce transient paralysis and spectacular behavioral manipulations in stung hosts resulting in suppression of escape reactions. It is said for example that a cockroach stung by *Ampulex compressa* "follows the wasp in a docile manner like a dog on a leash" (Libersat, 2003). In social Apidae and Vespidae, venom primarily serves as a defensive weapon against predators and competitors (Piek, 1986). These venoms are generally algogenic (pain inducers) to vertebrates (Chen and Lariviere, 2010) and some arthropods (Eisner and Camazine, 1983). They can produce local damage and allergic reactions in large vertebrates and may be lethal to invertebrates and small vertebrates. On contrary to Terebrantia species, the venom composition in social Apidae and Vespidae is more homogeneous at the genus scale (Hoffman, 2006). Ant venoms display the greatest diversity of biological roles in being either used as injected or topically applied defensive secretions, offensive weapons for the capture of prey, trail, sex, aggregation and alarm pheromones, or repellents (Schmidt, 1986c). Their venom composition also greatly differs from what is observed in other families of Hymenoptera. The analysis of venoms from eight species representative of Myrmicinae, Paraponerinae and Ponerinae subfamilies showed they contained fewer glycoproteins and more acidic proteins than venoms from social Apidae and Vespidae (Leluk et al., 1989). The venoms of some Myrmicinae (e.g. in *Solenopsis* and *Monomorium* genera) contain up to 90–95% N-alkyl and alkenyl-piperidine alkaloids and a small fraction of allergenic proteins (Jones et al., 2003; Hoffman, 2006). Venoms from ant subfamilies Paraponerinae, Ponerinae, Ectatomminae, Pseudomyrmeciinae, Myrmeciinae, and Myrmicinae are particularly rich in peptides with neurotoxic, myotoxic, cytotoxic or antimicrobial properties (Orivel et al., 2001; Inagaki et al., 2004; Johnson et al., 2010; Szolajska et al., 2004). In Formicinae and Dolichoderinae subfamilies, the aculeus has regressed and has lost its stinging function. The venom of Formicinae is mainly composed of formic acid and contains <5% amino acids and small peptides. During fights, the venom is mixed with air and secretions from Dufour's gland and sprayed on the opponents (Schmidt, 1986c).

Besides these well-studied venomous functions, the antimicrobial properties of hymenopteran venoms have often been considered of secondary importance albeit it constitutes the unique venomous function broadly distributed among distant hymenopteran species. It is widely argued that the adaptive significance of this trait relies on the avoidance of the contamination of the venom apparatus by opportunistic pathogens, contracted at the occasion of stinging events. However it has never been proven. In addition, the recent discovery that venoms are also used in collective and social immunity contexts in bees and perhaps in a social paper wasp (Turillazzi et al., 2007; Baracchi and Turillazzi, 2010; Baracchi et al., 2011) throws into question the biological, ecological and evolutionary implications of this function: against which categories of microbes these venoms are active in the wild? Why and how? Does the production of venom antimicrobial molecules result from independent convergent evolution or from the conservation of ancestral properties? Is there any link between the antimicrobial properties of venoms and the other venomous functions? In the following sections I will discuss these original aspects of the venomous function. I hope it will "pique" your interest.

3. Hymenoptera stings and infections

3.1. Do Hymenoptera of medical importance transmit infectious diseases through their sting?

Reactions of vertebrate organisms, notably humans, to stings of social Hymenoptera have focused the attention of many research-

ers and have been extensively reviewed (Schmidt, 1986a; Antonicelli et al., 2002; Ebo et al., 2005; Bircher, 2005; Biló et al., 2005; de Graaf et al., 2009). Main species of medical importance belong to families of social Apidae (genera *Apis* and *Bombus*), Vespidae (genera *Polistes*, *Vespula*, *Dolichovespula* and *Vespa*) and Formicidae (genera *Myrmecia*, *Tetramorium*, *Pachychondyla*, *Solenopsis*, *Pogonomyrmex* and *Formica*) (Klotz et al., 2009). To a lesser extent, some consequences (e.g. pain induction) of rare accidental envenomations of humans by other Formicidae and solitary Scoliidae, Pompilidae, Mutillidae, Sphecidae and Anthophoridae have also been documented (Schmidt, 1986b and Schmidt, 1986c; Fukuzawa et al., 2002; Rodriguez-Acosta and Reyes-Lugo, 2002; Bircher, 2005; Haddad Junior et al., 2005).

The main threat related to these species concern the induction of serious reactions in hyper-reactive or hypersensitive patients. Venom hypersensitivity covers venom allergy and nonallergic venom hypersensitivity (Johansson et al., 2001). The former may be mediated by IgE isotype antibodies or by non-IgE immunologic mechanisms involving IgG isotype antibodies and/or mediators released by immune cells such as mast cells (Fricker et al., 1997; Biló et al., 2005). Venom-induced anaphylaxis is most often mediated by IgE (Biló et al., 2005). A single sting can induce immediate or delayed hypersensitive reactions up to fatal anaphylaxis (Table 1). Stings of Hymenoptera of medical importance are characterized by the injection of large quantities of venom proteins and allergens. Apinae and Bombinae stings release the highest amounts of venom proteins. Vespinae inject less venom protein but are capable of repeated stings (Hoffman and Jacobson, 1984; Mulfinger et al., 1992). Most species of medical importance produce several venom allergens which together represent an important proportion of their venom proteins. In *A. mellifera* for instance, 12 venom proteins including major venom components are potentially antigenic to humans. The amount of venom allergens injected through a single sting is thus comparable to some of the highest doses of pollen allergens inhaled annually (Takahashi et al., 2007). Venom phospholipases A1B and A2 (PLA2), hyaluronidases, acid phosphatases, proteases and antigen 5 family members account for the main allergens identified to date from Hymenoptera venoms (de Graaf et al., 2009). Other species-specific allergens are also known such as melittin and Api m6 from *A. mellifera*, Sol i 2 and 4 from the fire ant *Solenopsis invicta* and Myr p 1 (=pilosulin 1), 2 (=pilosulin 3) and 3 (=pilosulin 4) from the jumper ant *Myrmecia pilosula* (Donovan et al., 1996; Wu et al., 1998; Hoffman, 2006). In addition, honeybee and vespid venoms may cross-react with non-related plant glycoproteins and also with each other, by means of allergenic carbohydrate epitopes (Hemmer et al., 2001; Antonicelli et al., 2002).

Table 1
Main clinical symptoms reported in humans upon stings of social Hymenoptera.

Reactions	Symptoms
Immediate	
Small and local	Pain
	Pruritis
	Urticaria
Large and local	Edema
	Erythema
Mild and systemic	Urticaria
	Angioedema
	Erythema
	Pruritis
Moderate and systemic	Urticaria
	Mild respiratory symptoms
	Dizziness
	Nausea and vomiting
Severe and systemic	Laryngeal edema
	Bronchospasm
	Hypotension
	Shock
	Loss of consciousness
	Upper airway edema
Delayed	Serum sickness
	Vasculopathy
	Arthralgia
	Encephalopathy
	Neuritis
	Renal disease
	Liver necrosis

Table adapted from Schmidt (1986a), Reisman (1994), Koçer et al. (2003), Biló et al. (2005) and de Graaf et al. (2009).

All these species belonging to the Aculeata superfamily (approx. 92,000 species), the first good news at this stage is that more than two-third of the estimated total number of hymenopteran species (280–320,000 species) (Pennacchio and Strand, 2006) are potentially not noxious toward us. The second good news is that no social species of medical importance seems to be able to directly transmit infectious diseases to us through their sting, although numerous pathogenic viruses, bacteria, protozoans and fungi have been described in social Hymenoptera (Schmid-Hempel, 1998; Viljakainen et al., 2009). A similar comment applies to solitary aculeates which do not figure among known vectors of borne diseases. This finding seems obvious but merits attention: It is in sharp contrast with well-known cases of biting and unloved arthropods (mosquitoes, sandflies, tabanidae and simuliidae flies, fleas, ticks, body lice…) which make excellent vectors of viruses, bacteria, protists or nematodes (Gratz, 1999). Also note that affection is a bad advisor in this domain, since even a lovely cat can potentially transmit at least 9 different zoonotic agents to us through occasional bites and scratches (Brown et al., 2005) and that 28–80% of cat bites become infected (Talan et al., 1999). There is still time to get rid of it.

The intrinsic antimicrobial properties of aculeate venoms, notably relying on the confirmed presence of venom antimicrobial peptides (AMPs) in many species may intuitively explain this apparent absence of disease transmission. However, this very credible hypothesis has not yet been tested experimentally *in vivo* and is not the only possible explanatory variable. Other possible assumptions can be summarized as follows:

1. Social insects have evolved many colony-level defenses against pathogens including hygienic behaviors, co-adaptation between host colonies and vertically transmitted mutualists, incorporation of antimicrobial compounds into nest materials and stored food, and adaptation of queens' reproductive strategies to increase genetic diversity within colonies (Fujiwara et al., 1990; Viljakainen et al., 2009; Kwakman et al., 2010; Armitage et al., 2011). In some species, venom seems to play a preventive role in being sprayed on the surface of juveniles which would avoid horizontal transmission of pathogens (Obin and Vander Meer, 1985; Baracchi and Turillazzi, 2010; Baracchi et al., 2011). These mechanisms of collective and social immunity supplement individual defenses and strongly constrain the acquisition, resilience and transmission of parasites and pathogens within hymenopteran colonies (Evans et al., 2006).
2. Contrary to biting arthropods, most, if not all, Hymenoptera do not need to contact vertebrates to survive or to allow their progeny to develop. The defensive function of their venoms, notably based on pain and cytotoxicity induction, even tends to decrease the probability of contacts occurring. As such, the dissuasive role of these venoms could also be interpreted as a component of collective or social immunity.
3. Some venom allergens display cytolytic and antimicrobial activities (e.g. bee melittin and PLA2). A possible connexion between induction of local or systemic hypersensitive reactions, venom

antimicrobial components and absence of disease transmission can thus be envisaged but remain to be investigated both at epidemiological and physiological levels.

4. The loss of use of the ovipositor for oviposition in most of the Aculeata reduces the sources of possible infected secretions delivered to stung individuals since the stinging organ only serves to inject products from the venom apparatus and the Dufour's gland and not from the reproductive organs. This is in sharp contrast to the situation of Terebrantia parasitic wasps, whose higher potential for pathogen transmission have been thought to be used to control pest insects (Quicke, 1997).
5. Hymenoptera use their venom to defend themselves against a wide range of encounters, but most frequently, they use it against other arthropods. Even if some vertebrates' pathogens would be able to circulate into groups of social Hymenoptera or populations of solitary species, their transmission through stings to a suitable intermediary or definitive vertebrate host would thus be rather hazardous and hence reproductively costly. In addition, stings by social Hymenoptera are often lethal to invertebrates, probably making stung animals poor quality and too short-lived hosts for circulating parasites and pathogens.

Hymenoptera of medical importance seem thus to constitute suboptimal vectors for agents of borne diseases affecting vertebrates. The features of venom and venom producing organs likely also contribute to limit microbial development and transmission and this may not solely concern species of medical importance.

3.2. Can microbes successfully develop within venom apparatuses of Hymenoptera?

3.2.1. Fungi

Examples of fungal species successfully colonizing the venom gland and reservoir of Hymenoptera are rare. Three cases of "symbiotic associations" between parasitic Hymenoptera (*Comperia merceti*, *Dapsilarthra apii* and *Pimpla turionellae*) and vertically transmitted "yeasts" have been reported by Quicke (1997). I have chosen to discuss these examples below because they shed an interesting light on the question addressed in this section.

In *C. merceti* (Encyrtidae), a yeast-like organism related to *Candida* species (Saccharomycotina) was found in the venom reservoir of adult females (LeBeck, 1985). In its initial description, LeBeck stated that the fungus was injected along with venom into eggs of the host cockroach *Supella longipalpa*. The author suggested a possible symbiotic relationship between the fungal microorganism and *C. merceti* in which the venom reservoir could constitute a transmission organ. However convincing evidence for such a scenario is still lacking. Firstly, the yeast exhibits a relatively wide tissue distribution. In parasitized cockroach, it was detected in the degenerating embryonic tissues. It was also observed in the gut and hemolymph of developing parasitoid larvae and adults of both sexes, and in the fat body of adult males and females (LeBeck, 1989). *C. merceti* being gregarious, transmission to offspring is in fact both vertical (from infected parasitoid females to their offspring, via penetration through the egg's surface) and horizontal (probably from infected host eggs to offspring of uninfected parasitoid mothers) (Gibson and Hunter, 2009). Secondly, in the venom reservoir, all yeast cells are present within an additional inner membrane whose histologic origins and function(s) remain unclear (LeBeck, 1989). Intriguingly the author stated that the relative concentrations of yeast per venom reservoir were not different between newly emerged females and females allowed to oviposit for various lengths or time. Whether this membrane allows the passage of yeast cells to inoculate host eggs during oviposition is thus not certain. On the other hand, LeBeck was somehow evasive on the possible presence of yeast cells within the oviduct and secondary glands of the female's genital tract ("yeast were not expelled from secondary accessory glands during dissections because the thick gland reservoir walls did not easily rupture") (LeBeck, 1989). These organs may also represent potential reservoirs for the parasite. More importantly, the fungus appears to play no role in the parasitic relationship and even induces significant fitness costs to the infected wasps (Gibson and Hunter, 2009). Fungus-free strains have been obtained in the laboratory, showing that the association is facultative. Gibson and Hunter (2009) have hypothesized that it could have evolved from a fungal symbiont of host cockroaches and may thus be considered as a mutualist of the cockroaches. Given the induced fitness costs and the apparent absence of benefits of this association for the wasp, one could go further and definitely consider it as a facultative parasite of the parasitoid rather than a mutualist, as initially supposed by LeBeck.

A parasitic yeast-like fungus was observed multiplying in the hemolymph of mining larvae of the celery fly *Philophylla heraclei* parasitized by *Dapsilarthra* (=*Adelura* = *Alysia*) *apii* (Braconidae) (Keilin and Tate, 1943). It was hypothesized to be transmitted from parasitoid females to hosts during oviposition but its ability to colonize the venom apparatus or genital tract of *D. apii*, if evaluated, was not reported. It would be interesting to re-investigate this biological system with contemporary means both for fundamental purposes and to improve the biocontrol of the celery fly.

Females of the Ichneumonidae *P. turionellae* host and transmit a commensal, barely parasitic, endosymbiont (Middeldorf and Ruthmann, 1984) initially reported as a yeast-like microorganism, but whose ultrastructural features are strongly evocative of a *Nosema*-like microsporidia. The tissue distribution of spores and primary stages of the parasite encompasses ovaries (ovocytes and ovarian cells), fat body, gut and malpighian tubules, gut muscles and nervous tissues (Pavenstädt-Grupp and Ruthmann, 1989). This widespread distribution partly originates from the transovarian passage of the parasite and its spreading during embryogenesis. Remarkably, heavy infection of the Dufour's gland was noted but the level of infection of the venom apparatus, again, was not reported. This suggests that the venom gland tubules and reservoir of *P. turionellae* would be minimally or less affected by the fungal parasite. Numerous other hymenopteran species, notably in Apidae and Vespidae families, can be infected by *Nosema* parasites (Sprague, 1978) or other microsporidia (Franzen et al., 2006) but it is uncertain whether these parasites frequently infect the venom apparatuses of their hosts. Recently, Copley and Jabaji (2011) have detected by duplex qPCR the presence of two microsporidia species (*Nosema ceranae* and *Nosema apis*) in the venom apparatus of *A. mellifera* foragers from Canadian hives. These obligatory intracellular fungal parasites are causal agents of Nosemosis, a serious disease affecting bee hives throughout the world. Both species were also detected in intestinal tract, hypopharyngeal glands, thoracic salivary glands and mandibular glands of adult bees, suggesting that bee glands may constitute seasonal reservoirs for spores of the parasites. Since the authors did not separate venom sacs from venom glands during their investigations, it is still unclear whether these parasites are also present in the venom of infected foragers. It is also not known whether the observed tissue distribution is representative of classical infections by *Nosema* species or corresponds to an atypical pattern resulting from local adaptations.

Schmid-Hempel (1998) has reported earlier observations of a fungal parasite, perhaps from the genus *Aspergillus*, causing melanosis-like symptoms in honeybee queens and which could successfully infect their venom sac and gland. This author also mentioned that *Saccharomycopsis* (=*Yarrowia* = *Candida*) *lipolytica* can infect the reproductive tract and venom apparatus of honeybee queens. Yet, honeybee venom displays antifungal activity at least towards certain fungal species (Yu et al., 2012). These infections seem

rather opportunistic and would mainly appear when queens are stressed or when their venom glands naturally degenerate after mating (Roat et al., 2006).

These few examples suggest that infections of the venom apparatus of Hymenopteran species by fungal parasites exist but are rather uncommon. When they occur, a widespread tissue distribution of parasites is often observed, which indicates the absence of a particular tropism towards such organs and the possible need of a general weakening of the organism to allow the development of fungi into the venom apparatus of infected individuals.

3.2.2. Bacteria

No examples of successful development of bacteria or eukaryotic organisms other than fungi seem to have been reported so far in the venom apparatuses of Hymenoptera species. Yet this set of organs, which are of ectodermic origin (Edson and Vinson, 1979), is potentially exposed to external contaminations. The presence of a valve at the basal opening of the venom reservoir is not systematic within Hymenoptera (Quicke, 1997). Hence, the lumen of venom reservoirs and associated glands directly communicates with the exterior in numerous species. The absence in venom glands of a bacterial *microbiota* strikingly contrasts with what is observed for instance, in the gut of numerous insects (Ryu et al., 2010; Hongoh, 2010) or in other exocrine glands of some Hymenoptera. For example, two mutualistic *Staphylococcus* species (*Staphylococcus arlette* and *Staphylococcus cohnii*) are hosted by the silk gland of pupating hornet larvae (Ishay et al., 2003). The bacteria are secreted with silk, then partially digest parts of the silk weave and facilitate the egress of the imago from the puparium. *Streptomyces* bacteria were found in antennal glands of female beewolves (solitary digger wasps, Crabronidae family) within the genera *Philanthus*, *Philantinus* and *Trachypus* (Kaltenpoth et al., 2012). These symbiotic bacteria are transmitted to developing larvae which latter transfer them to their cocoons where they provide effective defense against mould development. Finally, some fungus-growing ants (Attini, Myrmicinae subfamily) could have cuticular structures potentially associated with glands that produce nutrients for the growth of their mutualistic *Actinomycete* bacteria (unpublished data cited in Currie, 2001).

On the other hand, DNA fragments of *Wolbachia* endosymbionts have recently been amplified from venom gland cells of the Iranian scorpion *Hemiscorpius lepturus* (Baradaran et al., 2011) and from salivary gland cells of *Aedes albopictus* (Zouache et al., 2009). It would be surprising that no bacterial exo- or endosymbionts could be able to similarly colonize venom gland cells of Hymenoptera. In any case, it is tempting provisionally to state that venom producing and storing organs of healthy individuals do not constitute suitable environments for the development of fungal, protozoan or bacterial microbes. The presence of viruses, however, is far from being excluded.

3.2.3. Viruses

It is now established that venom glands and ovaries of Terebrantia parasitoid wasps may contain a great diversity of free viruses (Renault, 2012; Varaldi et al., 2012). They may belong to Ascoviridae, Poxviridae, Reoviridae, Rhabdoviridae, Coronaviridae, picorna-like viruses or to yet unclassified groups of viruses. However, these associations are mostly facultative and do not always result in the secretion of viral particles in the venom gland lumen.

A pathogenic picorna-like virus (PpSRV) was detected in the cytoplasm of secretory cells of abnormal venom apparatuses of *Pteromalus puparum* females (Pteromalidae) (Zhu et al., 2008). The prevalence of this infection in adults of the *P. puparum*'s population surveyed by the authors was about 5% and was stable over a 2 year period. The ability of infected females to produce venom was not reported but was likely to be compromised in view of the abnormal morphology of infected venom apparatuses.

A corona-like virus, PcCo-likeV, was purified from the venom apparatus of the braconid wasp *Psyttalia* (=*Opius*) *concolor* (Jacas et al., 1997). Its localization in other organs of the wasp, mechanisms of infection and potential involvement in host-parasitoid interactions are unknown. According to the authors, corresponding viral particles were found freely within the lumen of the venom gland. Nevertheless this latter localization needs to be confirmed since there is no firm evidence that the observed particles could be attributed to PcCo-likeV rather than to another virus (e.g. to an associated reovirus) (Renault, 2012). In another *Opius* species (*Opius caricivorae*), similar virus-like particles were observed in ovarial epithelial cells and secretory cells of venom gland filaments, but not in the reservoir of the venom apparatus (Wan et al., 2006). The viral nature of these particles has not been established yet. In contrast, one member of Rhabdoviridae (DlRhV) has been detected in distal cells of the venom apparatus of the braconid *Diachasmimorpha* (=*Biosteres* = *Opius*) *longicaudata* (Lawrence and Akin, 1990; Lawrence and Matos, 2005) and would be secreted into the venom. DlRhV would infect the wasp's offspring during oviposition, probably *via* micropylar openings at the surface of oviposited eggs and is injected into *D. longicaudata*'s host. In the wasp, DlRhV is always found in association with the entomopoxvirus DlEPV. DlEPV occupies a different zone of the venom apparatus and is also injected into the host during oviposition (reviewed by Renault, 2012). The rhabdovirus DlRhV could accumulate in cell epidermis of the host hence blocking its molting process to the benefit of developing parasitoid eggs (Lawrence, 1988). In addition, DlEPV triggers apoptosis of host hemocytes, thus impairing its ability to mount an efficient encapsulation response toward eggs of *D. longicaudata* (Lawrence, 2005).

In *Diadromus pulchellus* (Ichneumonidae), the reovirus DpRIV-1 is also found, among other tissues, in venom glands of adult females (Rabouille et al., 1994). DpRIV-1 has no impact on the fitness of the wasp and is transmitted to pupae of the lepidopteran host *Acrolepiopsis assectella* where it remains unamplified for several days. In the host, DpRIV-1 regulates the replication of the associated ascovirus DpAV-4, present in the genitalia of the female wasp and co-injected during oviposition (Bigot et al., 1997). DpAV-4 inhibits the melanization of cellular capsules mounted by host pupae against implanted foreign bodies (Renault et al., 2002). Therefore, DpAV-4 may contribute to the parasitic success of *D. pulchellus* when its replication is regulated by DpRIV-1.

The venoms from a number of *Leptopilina* endoparasitoid species (Figitidae) and from the braconid endoparasitoid *Meteorus pulchricornis* are also known to contain virus-like particles (VLPs) (Suzuki et al., 2008; Gatti et al., 2012). These VLPs actively participate in suppression of host immunity in selectively destroying subpopulations of hemocytes involved in encapsulation of foreign bodies or in reducing their spreading abilities (Rizki and Rizki, 1990; Morales et al., 2005; Suzuki and Tanaka, 2006). However, in contrast to free viruses, VLPs present in these venoms would be devoid of encapsidated nucleic acid and their supposed viral origins are still a matter of debate. Very recently, the new term of "venosome", less ambiguous and polysemantic than VLP, has been proposed to design these membrane vesicles when they are produced by venom glands (Gatti et al., 2012). In any case, venosomes cannot be considered as truly forms of mutualistic organisms able to colonize venom apparatuses as for DlEPV or DpAV-4. At the most and provided this shall be proven 1 day, they would only constitute the highly selected remains of an ancient association between some parasitic wasp lineages and hypothetical ancestral viruses.

In summary, can we state that venom apparatuses of Hymenoptera are suitable organs for the development of viruses but not for

other microbial agents? A general pattern is difficult to extrapolate from these few examples: Firstly, the composition and complexity of venom greatly varies between hymenopteran species, especially among Terebrantia parasitoids (Moreau and Guillot, 2005; Formesyn et al., 2012) and ants (Hoffman, 2010). In consequence these secretions do not constitute homogenous biochemical environments and in some cases the venom clearly appears to possess antiviral properties. This is notably the case in honeybee, in which melittin, the major venom component, displays antiviral properties against murine retroviruses, tobacco mosaic virus, herpes simplex virus and HIV-1 (reviewed in Wachinger et al., 1998). On the other hand, the presence of microbes in venom or venom-secreting cells may have been overlooked in numerous cases, particularly for endosymbionts. However it seems proven that the venom apparatus of a number of solitary parasitic Hymenoptera may constitute a permissive environment for the replication, persistence and/or transmission of some pathogenic (e.g. PpSRV), commensal (e.g. DpRIV-1) or even mutualistic (e.g. DlRhV, DlEPV, DpAV-4) viral agents. This has to be put in perspective with the fact that, in solitary endoparasitoids belonging to the Braconidae family, venoms facilitate the action of symbiotic PDVs produced by the females' ovaries. PDVs cause endocrine dysfunction and immunosuppression in parasitized hosts and their action is either independent or strongly dependent on the presence of venom (Beckage and Gelman, 2004; Asgari, 2006, 2007; Moreau et al., 2009a). The exact mechanism of synergy between venom and PDVs is not known but venom may stabilize PDV particles in the host and may facilitate their entry into host's cells (Asgari and Rivers, 2011). For instance, in *Cotesia melanoscela* (Braconidae) venom enhances the uncoating of PDVs at nuclear pores in host's hemocytes (Stoltz et al., 1988). In *Cotesia rubecula*, a 1.6 kDa venom peptide (Vn1.5) is essential for expression of PDV genes into host's tissues (Zhang et al., 2004). However, in a third *Cotesia* species (*Cotesia congregata*) venom is not required for the entry and expression of the associated PDV (Beckage et al., 1994). The intrinsic properties of venom, along with specificities in the *modus operandi* of viruses, appear thus as key determinants of the level of tolerance, synergy or even dependence between venom secretions and viruses. Otherwise, the venoms of Hymenoptera appear rather hostile to the development of other types of microbial parasites or pathogens and as observed so far, do not seem to host mutualistic bacteria.

3.2.4. Factors contributing to limit infections of venom apparatuses in Hymenoptera

What physiological and biochemical features could lead to this apparent situation? Intuitively, the relative sterility of venom apparatuses may at first rely on the presence of antimicrobial molecules in the venom. The argument is often advanced to propose with minimal efforts a function for newly described venom AMPs (the numerous references including this assumption were intentionally removed from these brackets so as to avoid prosecution). Note that this hypothesis has never been tested *in vivo* or on isolated organs and is worthy of further investigation, for example with methods of gene silencing now available for hymenopteran models (Hunter et al., 2010). Rightly or wrongly, this assumption is provisionally weakened by the fact that very few antimicrobial molecules, notably AMPs, have so far been characterized in venoms from parasitoid Hymenoptera (Table 2). Yet they are also apparently devoid of an *intravenenum microbiota*. This suggests that other influencing elements may also have to be considered. Physico-chemical features of venom secretions (pH, osmolarity and metabolites concentrations different from other biological fluids) and abundant presence of various hydrolases may considerably affect the survival of microbes in venom. In addition, in most hymenopteran venom apparatuses, the lumen of venom glands and reservoir is internally lined with a thin epicuticle. The cuticular lining is prolonged up to intracellular secreting organelles (also named end apparatus) of secretory cells *via* thin ductules formed by duct cells (Bridges and Owen, 1984; Jiménez Peydró et al., 1996; Vårdal, 2006; Ferrarese et al., 2009; Moreau et al., 2009b). This histologic organization is generally interpreted as a physical barrier protecting secretory and epithelial cells from the venom toxins. A proteomic analysis of honeybee foragers' venom gland has confirmed that secretory cells do not appear particularly stressed, which provides evidence that this physical protection may be efficient (Peiren et al., 2008). It is reasonable to speculate that this epicuticular lining may, in addition, limit the propagation of potentially invading microorganisms and may block their access to nutrients. Interestingly, recent molecular investigations of venom gland products have identified several chitin-binding proteins in distant hymenopteran species (*Nasonia vitripennis*, *A. mellifera*, *Chelonus inanitus*) (de Graaf et al., 2010; Peiren et al., 2008; Vincent et al., 2010). Danneels et al. (2010) have suggested that in *N. vitripennis*, such proteins could both represent structural components of the epicuticular lining of venom glands and possess an antibacterial function. Under such drastic living conditions, size might matter. The small size of viral particles could be a major asset to exploit cuticular breaches and interruption of cuticle lining in the vicinity of intracellular end apparatus of venom gland secretory cells. Further investigation is necessary to unravel the possible relationship between structural and biochemical features of the venom apparatus and how it limits colonization by microorganisms.

Venoms of the Hymenoptera may not only contribute to the relative sterility of the stinging apparatus. They also likely contribute to controlling infection by opportunistic pathogens in stung individuals.

3.3. Do secondary infections occur after hymenopteran stings?

If we assume that the venom apparatuses of Hymenoptera may be unsuitable to the survival of pathogenic microorganisms, with the exception of viruses and some fungal parasites, then any new reaction caused by other microbes and occurring as a direct consequence of a sting may be indicative of a secondary infection. Opportunistic pathogens may originate from the abdomen of stinging Hymenoptera, from the external surface of stung animals (skin or cuticle) or from their immediate environment. They may also correspond to pre-established microorganisms maintained at non-pathogenic thresholds by physical barriers and constitutive immune defenses until the stinging event. In theory, evidence of superinfection in relation to a sting would include the observation of one or several of the following signs in stung animals: triggering of humoral and cellular antimicrobial responses (e.g. induction and overexpression of AMPs, activation of specialized immune cells and pro-inflammatory processes), development of pathological symptoms indicative of on-going infection in envenomated tissues, and ideally, direct detection of the presence of pathogens in these tissues and/or in circulating biological fluids. Ultimately, envenomation should increase the incidence of microbe-induced mortality among stung individuals as a result of unsuccessful control of the infection in a significant proportion of envenomated animals. I have sought these elements in past and recent literature. Whether venoms may counteract or facilitate secondary infections is another point that I will try to clarify, in relation to the biology of species cited as examples.

3.3.1. Parasitoid Hymenoptera

In an impressive table summarizing about 600 references, Piek and Spanjer (1986) have reported the paralytic and lethal potential of numerous solitary hymenopteran species. Only seven out of the 579 listed species potentially caused the death of invertebrate hosts and prey in natural conditions, as a direct result of the use

Table 2
Antimicrobial venom components and venom antimicrobial potentials characterized in solitary Hymenoptera.

Species	Active against	Inactive against	Notes	References
Ichneumonidae				
Pimpla hypochondriaca (Pimplinae, P)				
Venom	G− (Eco, **Xca**)	G− (Psy) G+(Bce, Bsu)		Dani et al. (2003)
Pimpla turionellae (Pimplinae, P)				
Venom		G8 (Eco) G+ (Sau) F (Cal)	Cytotoxic and cytolytic toward insect cultured cells	Ergin et al. (2006)
Braconidae				
Cotesia congregata (Microgastrinae, P)				
Venom		G− (Eco) G+ (Sau)		Chevignon, Moreau et al. (unpublished data)
Pteromalidae				
Pteromalus puparum (Pteromalinae, P)				
PP13	G− (Eco) G+ (Bsu, Bpu, Sau, **Slu**)	F (Bci)	Cationic linear α-helical peptide, not hemolytic, salt-sensitive	Shen et al. (2009)
PP102	G+ (Bsu, Bpu, **Sau**, Slu)	G− (Eco) F (Bci)	Cationic linear α-helical peptide, not hemolytic, salt-sensitive	Shen et al. (2009)
PP113	G+ (Bsu, Bpu, **Sau**, Slu)	G− (Eco) F (Bci)	Cationic linear α-helical peptide, not hemolytic, salt-sensitive	Shen et al. (2009)
Nasonia vitripennis (Pteromalinae, P)				
defensin-NV	G− (Eco, Pae) G+ (Bce, **Bdy**, Sau) F (Cal)		Sequence similarities with defensing 1–2 from *N. vitripennis*, weakly hemolytic	Ye et al. (2010)
Megachilidae				
Osmia rufa (Megachilinae, SH)				
Osmin	G− (Eco) G+ (Mlu) F (**Fox**)		Cationic amphipathic α-helical peptide, weakly hemolytic compared to bee mellitin	Stöcklin et al. (2010)
Apidae				
Melecta albifrons (Apinae, C)				
Melectin	G− (Eco, Pae) G+ (**Bsu**, Sau) V (HIV-1)		Cationic amphipathic α-helical peptide, weakly hemolytic, mast cells degranulating activity	Čeřovský et al. (2008) and Wang et al. (2010)
Vespidae				
Orancistrocerus drewseni (Eumeninae, SH)				
OdVP1 (=eumenine mastoparan-OD)	G− (Eco) G+ (Sau) F (Bci, **Cal**)		Cationic linear α-helical peptide, hemolytic, cytolytic toward insect cultured cells, low antibacterial activity	Murata et al. (2009), Baek and Lee (2010) and Baek et al. (2011)
OdVP2 (=orancis-Protonectin)	G− (Eco) G+ (Sau) F (**Bci**, Cal)		Cationic linear α-helical peptide, hemolytic, cytolytic toward insect cultured cells, low antibacterial activity	Murata et al. (2009), Baek and Lee (2010) and Baek et al. (2011)
OdVP3	F (**Bci**, Cal)	G− (Eco) G+(Sau)	Cationic linear α-helical peptide, cytolytic toward insect cultured cells, low antibacterial activity	Baek and Lee (2010) and Baek et al. (2011)
OdVP4		G− (Eco) G+(Sau) F (Bci, Cal)	Cationic linear α-helical or coil peptide	Baek and Lee (2010) and Baek et al. (2011)
Eumenes pomiformis (Eumeninae, SH)				
EpVP1	G− (Eco) G+ (Sau) F (**Bci**, Cal)		Cationic linear α-helical peptide, cytolytic toward insect cultured cells	Baek et al. (2011)
EpVP2a	G− (Eco) G+ (Sau) F (**Bci**, Cal)		Cationic linear α-helical peptide, cytolytic toward insect cultured cells	Baek et al. (2011)
EpVP2b	G− (Eco) G+ (Sau) F (**Bci**, Cal)		Cationic linear α-helical peptide, hemolytic, cytolytic toward insect cultured cells	Baek et al. (2011)

Table 2 (*continued*)

Species	Active against	Inactive against	Notes	References
EpVP3		G− (Eco) G+ (Sau) (Bci, Cal)	Cationic coil peptide	Baek et al. (2011)
EpVP3S		G− (Eco) G+ (Sau) F (Bci, Cal)	Cationic coil peptide	Baek et al. (2011)
EpVP4a		G− (Eco) G+ (Sau) F (Bci, Cal)	Cationic coil peptide	Baek et al. (2011)
EpVP4b		G− (Eco) G+ (Sau) F (Bci, Cal)	Cationic coil peptide	Baek et al. (2011)
EpVP5		G− (Eco) G+ (Sau) F (Bci, Cal)	Cationic coil peptide	Baek et al., 2011
EpVP6	G+ (Sau) F (**Bci**)	G− (Eco) F (Cal)	Cationic linear α-helical peptide, lowly cytolytic toward insect cultured cells	Baek et al. (2011)
Eumenes rubronotatus (Eumeninae, SH)				
Eumenitin	G− (**Eco**, Pae) G+ (Bsu, **Sau**, **Ssa**) Protist (Lma)	G− (Ecl, Pmi) G+ (Sau, Sep, Bth)	Cationic linear α-helical peptide, very weakly hemolytic, lower mast cells degranulating activity than EMP-AF and mastoparans, pore forming activity inhibited by the presence of cholesterol in membranes	Konno et al. (2006), Arcisio-Miranda et al. (2008), Dos Santos Cabrera et al. (2009) and Rangel et al. (2011)
Eumenes rubrofemoratus (Eumeninae, SH)				
Eumenitin-R	G− (Eco, Pae, Pmi, Sma) G+ (Bsu, Mlu, Sau, Sep, Spy) F (**Cal**) Protist (Lma)		Cationic linear α-helical peptide, weakly hemolytic, mid mast cells degranulating activity, very weak leishmanicidal activity	Rangel et al. (2011)
EMP-ER	G− (Eco, Pae, Pmi, Sma) G+ (Bsu, Mlu, Sau, Sep, Spy) F (**Cal**) Protist (Lma)		Cationic linear α-helical peptide, weakly hemolytic, mast cells degranulating activity	Rangel et al. (2011)
Eumenes fraterculus (Eumeninae, SH)				
Eumenitin-F	G− (Eco, Pae, Pmi, Sma) G+ (Bsu, Mlu, Sau, Sep, Spy) F (**Cal**) Protist (Lma)		Cationic linear α-helical peptide, weakly hemolytic, mid mast cells degranulating activity	Rangel et al. (2011)
EMP-EF	G− (Eco, Pae, Pmi, Sma) G+ (Bsu, Mlu, Sau, Sep, Spy) F (**Cal**) Protist (Lma)		Cationic linear α-helical peptide, weakly hemolytic, mast cells degranulating activity	Rangel et al. (2011)
Anterhynchium flavomarginatum micado (Eumeninae, SH)				
EMP-AF	G− (Eco, Pae) G+ (**Sau**, **Sep**, **Ssa**, Ssu) Protist (Lma)		Cationic linear α-helical peptide, similar to hornets' mastoparans, lowly hemolytic, mast cells degranulating activity comparable to mastoparans	Konno et al. (2000), Delatorre et al. (2001), Dos Santos Cabrera et al. (2004) and Rangel et al. (2011)

(*continued on next page*)

Table 2 (*continued*)

Species	Active against	Inactive against	Notes	References
EMP-AF1	G− (Eco, Pae) G+ (**Sau**, Sep, Ssa, Ssu)	F (Cal)	Analogue of EMP-AF with deamidated C-terminus, reduced antimicrobial potency compared to EMP-AF, lower mast cells degranulating activity than EMP-AF and EMP-AF2, not hemolytic	Konno et al. (2000) and Dos Santos Cabrera et al. (2004)
EMP-AF2		G− (Eco, Pae) G+ (Sau, Sep, Ssa, Ssu) F (Cal)	Shorter analogue of EMP-AF, mast cells degranulating activity, very weakly hemolytic	Konno et al. (2000) and Dos Santos Cabrera et al. (2004)
EMP-AF3		G− (Eco, Pae) G+(Sau, Sep, Ssa, Ssu) F (Cal)	Shorter analogue of EMP-AF with deamidated C-terminus, lower mast cells degranulating activity than EMP-AF and EMP-AF2, very weakly hemolytic	Konno et al. (2000) and Dos Santos Cabrera et al. (2004)
Oreumenes decoratus (Eumeninae, SH)				
Decoralin	G− (Eco, Kpn, **Afa**) G+ (**Bsu**, **Bth**, **Sau**, **Ssa**) F (Cal) Protist (Lma)	G− (Eco)	Cationic linear α-helical peptide, original antimicrobial activity lower than anoplin but much improved when C-terminus is amidated, weak mast cells degranulating activity, not hemolytic	Konno et al. (2007)
Pompilidae				
Anoplius samariensis (SH)				
Anoplin	G− (Eco, Pae) G+ (Bsu, **Sau**, **Ssu**)	G− (Ecl, Pmi) G+ (Bth)	Cationic linear α-helical peptide, similar to crabrolin and mastoparan-X, salt-sensitive, mast cells degranulating activity depending of the cell system used, weakly hemolytic, possesses ion channel-like activity on anionic bilayers. Probably one of the smallest natural AMP (only 10 amino acid residues long)	Konno et al. (2001), Ifrah et al. (2005), Dos Santos Cabrera et al. (2008, 2009) and Won et al. (2011a,b)

For each type of microorganisms (Gram-negative and Gram-positive bacteria, virus, fungi and protists), abbreviated names of tested species are given within brackets. Microorganisms against which a given venom or venom component has been found to be the most active are indicated in bold.
Abbreviated names for Gram-negative bacteria (G−): Afa, *Alcaligenes faecalis*; Ecl, *Enterobacter cloacae*; Eco, *Escherichia coli*; Kpn, *Klebsiella pneumonia*; Pae, *Pseudomonas aeruginosa*; Pmi, *Proteus mirabilis*; Psy, *Pseudomonas syringae*; Sma, *Stenotrophomonas maltophilia*; Xca, *Xanthomonas campestris.* Abbreviated names for Gram-positive bacteria (G+): Bce, *Bacillus cereus*; Bdy, *Bacillus dysenteriae*; Bsu, *Bacillus subtilis*; Bth, *Bacillus thuringiensis*, Bpu, *Bacillus pumilus*; Mlu, *Micrococcus luteus*; Sau, *Staphylococcus aureus*; Sep, *Staphylococcus epidermidis*; Slu, *Sarcina lutea*; Ssa, *Staphylococcus saprophyticus*; Ssu, *Staphylococcus subtilis*; Spy, *Streptococcus pyogenes*. Abbreviated names for Fungi (F): Bci, *Botrytis cinerea*; Cal, *Candida albicans*; *Fox, Fusarium oxysporum*. Protist: Lma, *Leishmania major*; P, parasitoid species; SH, solitary hunting species; C, cleptoparasitic species.

of their venom. According to these authors, many, but not all solitary Aculeata and Terebrantia possess a paralyzing venom whereas only a few of them possess a lethal venom. These latter includes *N. vitripennis* (pteromalid pupal ectoparasitoid), whose non-paralytic venom induces developmental arrest, metabolic alterations and ultimately death of *Sarcophaga* (=*Neobellieria*) *bullata* hosts (Diptera: Sarcophagidae) after an extended period of time (up to 60 days at 25 °C) (Ratcliffe and King, 1967; Rivers and Denlinger, 1995; Rivers et al., 2006).

In this biological system, there is no evidence that death could be caused by systemic uncontrolled infections resulting from the action of the venom, at least in laboratory conditions. On the contrary, activation of finely tuned apoptotic and oncotic pathways is likely responsible for the death of hosts at the end of the parasitic association (Danneels et al., 2010). Interestingly the venom selectively affects host cellular immune responses: in the hours following envenomation, the total number of circulating hemocytes strongly declines, plasmatocytes and granular cells lose their adhesive and spreading ability, all plasmatocytes dye, and melanization and clotting are retarded (Rivers et al., 2002). These inhibitory effects are permanent and seem to be essential for host-feeding adult females and developing larvae of *N. vitripennis*, otherwise impaired in their feeding behaviors by host's clotting and wound-healing processes. Cellular death is promoted *via* an oncotic lytic mechanism involving stimulation of G-protein dependent signal transduction pathways, mobilization of calcium from intracellular stores and elevations in cAMP (Rivers et al., 2005). Interestingly, this mechanism was found to be very similar to that promoted by mastoparan, a potent antimicrobial and cytolytic peptide from venoms of hornets and other social vespids (Nakajima, 1986), during apoptosis/oncosis of vertebrate immune cells (Rivers et al., 2005). A laccase and a calreticulin-like protein identified among the venom proteins of *N. vitripennis* could correspond to the main elicitors of cell death (Abt and Rivers, 2007; Rivers and Brogan, 2008; de Graaf et al., 2010; Danneels et al., 2010).

Danneels et al. (2010) have underlined that a complete suppression of *S. bullata*'s immune responses would be disadvantageous for *N. vitripennis*, as it would increase the risk of host infection by external microbes. Several lines of defense could have been selected, mitigating this threat and placing the function of venom in a central role:

1. The venom-induced cell death selectively targets cells involved in the melanization and coagulation responses. This leaves open the possibility that stung hosts could still be able to activate their own humoral antimicrobial defenses like the production of AMPs by fat body (Danneels et al., 2010). Such a situation has already been observed in larvae of the dipteran host *Drosophila melanogaster*, parasitized by *Leptopilina boulardi* (endoparasitoid Figitidae). In this biological system, two venom

proteins of *L. boulardi* (LbGAP and LbSPNy) specifically target encapsulation and melanization responses (Labrosse et al., 2005; Colinet et al., 2009) but parasitized hosts keep their ability to produce antibacterial and antifungal peptides (Coustau et al., 1996; Nicolas et al., 1996; Schlenke et al., 2007).

2. Ye et al. (2010) have recently characterized a defensin-like antimicrobial peptide (Defensin-NV) from the venom of *N. vitripennis*, which displays a broad antimicrobial activity and a weak hemolytic potential (Table 2). This venom constituent could play an important role in contributing to the sterility of the venom reservoir content and/or in immediately eliminating opportunistic pathogens inoculated during the envenomation.
3. Certain venom proteins from *N. vitripennis*, whose sequences are related to proteins involved in immune processes, could stimulate the antimicrobial immune response of the host (Danneels et al., 2010).

Paradoxically, although the venom of *N. vitripennis* is categorized as lethal, parasitoid adult females hence allocate a significant part of their resources to produce venom proteins which could contribute to the long-lasting preservation of their host. The ability of *N. vitripennis* to preserve its host from secondary infection seems a prerequisite for the full and sustainable exploitation of its resources. The same requirement might apply to many parasitoid species, whether they develop internally or externally to the host's body.

The antimicrobial potential of the venom from *Pimpla hypochondriaca* (ichneumonid pupal endoparasitoid) was examined and was found active against Gram-negative bacteria (Dani et al., 2003) (Table 2). The venom also displays a cytolytic and paralytic activity and, similarly to the venom of *N. vitripennis*, triggers oncotic and apoptotic cell death pathways in culture cells and in some hemocytic populations of its natural host, *Lacanobia oleracea* (Richards and Dani, 2007; Rivers et al., 2009). The action of venom results in a strong impairment of hemocyte behavior and function such as spreading, encapsulation of foreign objects and phagocytosis of the Gram-negative bacteria *Escherichia coli* (Richards and Parkinson, 2000). Dani et al. (2003) have suggested that the injection of venom antibacterial factors in the host may provide to the host and to the wasp's progeny a protection against opportunistic pathogens during a period when the host's hemocyte-mediated immune responses are compromised. The venom of another *Pimpla* species, *P. turionellae*, was also examined but did not exhibit significant antimicrobial properties (Ergin et al., 2006). Larvae from this species appear to be protected from microbial infection thanks to their anal secretions that possess antibacterial and antifungal activity (Führer and Willers, 1986; Ergin et al., 2006).

Fang et al. (2010) found that artificial envenomation of pupae of the lepidopteran host *Pieris rapae* with a mix of Sephadex beads and filtered venom from another Pteromalidae, the gregarious endoparasitoid *P. puparum*, decreased the transcript levels of potentially antimicrobial and non-self recognition molecules among many other gene products. Interestingly, these authors have also recently characterized a C-type lectin (Pr-CTL), mainly expressed in granulocytes of *P. rapae* and whose constitutive expression is up-regulated following wounding and bacterial or artificial immune challenges (Fang et al., 2011). A slight up-regulation of Pr-CTL occurs in *P. rapae* hosts naturally parasitized by *P. puparum*, but its expression is totally inhibited by injection of elevated doses of venom from the parasitoid. Since Pr-CTL gene expression influences the expression of downstream immune-related genes involved in antimicrobial activity, parasitized *P. rapae* larvae could conserve some ability to defend themselves against microbial pathogens. As for *N. vitripennis*, the venom of *P. puparum* itself could also have a protecting role towards opportunistic pathogens: three cationic linear α-helical peptides mainly active against Gram-positive bacteria (Table 2), have been characterized from the venom of this parasitoid by Shen et al. (2009). It would be interesting to evaluate the real ability of parasitized and envenomated hosts to respond to microbial challenges and to analyze this response at different time points.

In the wild, *D. melanogaster* larvae develop in decaying fruits and vegetables which provide food and habitat (Basset et al., 2000). Therefore, adult females of larval endoparasitoid species have to penetrate this contaminated medium with their ovipositor to parasitize buried *Drosophila* larvae, which may facilitate inoculation of opportunistic pathogens to parasitized individuals and/or to female wasps. In *D. melanogaster* larvae parasitized by *Asobara tabida* (solitary braconid endoparasitoid) two genes encoding peptidoglycan-recognition proteins and three genes coding for AMPs are significantly up-regulated during the first 6 hours after parasitization (Wertheim et al., 2005). In addition, the melanization cascade of host larvae is only minimally impacted by the parasitism while their cellular immune responses are stimulated (Eslin and Prévost, 1998; Moreau et al., 2000, 2003; Prévost et al., 2005). On the other hand, a recent analysis of a cDNA library constructed from *A. tabida* venom gland allowed the identification of genes encoding potential antimicrobial molecules (Vinchon, 2010). These include a secreted PLA2-like protein sharing significant sequence similarity with honeybee venom PLA2, a potent antibacterial and trypanocidal enzyme (Perumal Samy et al., 2006; Boutrin et al., 2008). Several venom AMPs are known to activate hymenopteran venom PLA2s: in *A. mellifera* and *Bombus* sp. these activating peptides correspond to melittin and bombolitin III, respectively (Mingarro et al., 1995; Signor et al., 1994). Interestingly, several transcripts coding for short peptidic sequences preceded by predicted signal peptides are also produced by *A. tabida* venom gland cells and may be worth further investigations. *D. melanogaster* larvae envenomated and parasitized by *A. tabida* thus appear to be able to efficiently mount combined humoral and cellular immune responses to control secondary infections.

At the transcriptional level, parasitism of *D. melanogaster* larvae by a north-American *L. boulardi* strain induces similar effects as those observed during parasitism by *A. tabida* (Schlenke et al., 2007). In this association, parasitized hosts up-regulate a high proportion of Toll, JAK/STAT, and PO pathway genes within 12 h post-parasitization while Imd, JNK, and other *Drosophila* immune pathways appear weakly affected by the parasitism. These results validate earlier experiments showing that diptericin, which is under the control of the Imd pathway, is not expressed in wild-type *D. melanogaster* larvae upon parasitization by *L. boulardi* (Benassi et al., 2000). Interestingly, the Toll-regulated Drosomycin and Metchnikowin promoters, which both control the expression of antifungal peptides (Fehlbaum et al., 1994; Levashina et al., 1995; Broderick et al., 2009), are activated in the fat body of parasitized *D. melanogaster*. It is still unclear whether these transcriptional effects are promoted by the venom alone or in association with other maternal factors. At the cellular and humoral levels, the venom of *L. boulardi* has a more marked impact than that of *A. tabida*: it partially blocks the induction and release of lamellocytes from the lymph gland (main haematopoietic organ of host larvae), alters the morphology of circulating lamellocytes and inhibits the activation of proPO (Russo et al., 2001; Rizki and Rizki, 1992; Labrosse et al., 2003, 2005; Colinet et al., 2009). *L. boulardi* appears to invoke a complete immune response in *D. melanogaster* larvae that is only impaired at the final stage and which probably makes it possible for parasitized hosts to defend themselves against septic injuries. By contrast, parasitism of *Drosophila* larvae by another *Leptopilina* species, *L. heterotoma*, specifically blocks Toll pathway signaling in the fat body and severely impacts host hemocytes (Schlenke et al., 2007). The venom of this species causes the lysis of lamellocytes and the apoptosis of pro-hemocytes in the

lymph gland (Rizki and Rizki, 1994; Chiu and Govind, 2002). The impairment of the Toll pathway by venom and perhaps other virulence factors produced by *L. heterotoma* females may facilitate the avoidance of encapsulation and melanization reactions by parasitic eggs but may also strongly reduce the resistance of parasitized hosts to secondary infections. *L. heterotoma* partly compensates the side-effects of the high immune-suppressive abilities of its venom by parasitizing a broader host range than *L. boulardi* and by achieving higher successful parasitization rates in suitable hosts (Godfray, 1994).

In summary, there is no clear evidence that the stinging activity of parasitoid Hymenoptera results in a higher prevalence of secondary infections in their hosts or facilitates such infections. On contrary, in several biological systems studied, parasitoids appear to have evolved prophylactic venom-based strategies that limit the opportunity for pathogens to establish a secondary infection in their hosts. These may include the production and injection of venom antimicrobial proteins and peptides, the selective targeting of mediators of encapsulation and melanization reactions and the potential stimulation of the host's antimicrobial immune responses. These strategies are probably costly for parasitoids but may be adaptive in allowing a trade-off between avoidance of anti-parasitic immune responses and maintenance of the host's antimicrobial defences. One of the next challenges of the field will be to investigate the occurrence and epidemiology of natural pathogens in wild host-parasitoid systems in order to estimate the efficiency of venom-based antimicrobial strategies in such models.

Physiological adaptations are not the only way parasitoid wasps can avoid inoculating their hosts with opportunistic pathogens. The stinging behavior exhibited by some parasitoid females may also limit transmission of pathogens to the host of their progeny. For instance, McLaughlin and Adams (1966) performed small scale experiments suggesting that the speed of execution of the paralyzing sting by *Bracon mellitor* females (Braconidae) (soberly qualified as "very rapid"), could prevent the inoculation of *Mattesia grandis* (pathogenic Neogregarinida) to larvae of the boll weevil, *Anthonomus grandis*. Despite the fact that the pathogen could be found on the wasp ovipositor, it was almost never transmitted during oviposition to host larvae or to wasp progeny, even by heavily infected females. It would be interesting to test experimentally if a correlation does exist between the probability of pathogen transmission and the time elapsed during oviposition, which ranges in extreme cases (e.g. in rhyssine Ichneumonids) from 7 min to more than 5 h (Quicke, 1997).

3.3.2. Solitary predatory Hymenoptera

Many solitary aculeate species develop as provisional predators (Weaver et al., 2001) that transiently or permanently paralyze their prey before killing them. They either belong to the superfamilly Bethyloidea, Scolioidea, Pompiloidea, Sphecoidea, Apoidea or Vespoidea (Piek and Spanjer, 1986). Numerous authors reported the remarkable state of freshness and conservation of permanently paralyzed and eventually dead prey, even several weeks or months after the initial sting. It strongly suggests that distant groups of solitary wasps may have evolved conservation mechanisms to ensure the long-lasting preservation of stung prey. The most extensive recent works have been performed on biological activity and structural features of venom AMPs from solitary potter wasps (Vespidae, Eumeninae) (reviewed in Rangel et al., 2011) (Table 2). However, the biological roles these molecules really play in the wild are often overlooked in literature.

Konno et al. (2000) have first described Eumenine mastoparan-AF (EMP-AF), the major peptide component from the venom of *Anterhynchium flavomarginatum* micado. EMP-AF has structural features similar to MCD peptides of the mastoparan family and similarly adopts an amphipathic α-helical conformation in anisotropic or membrane mimetic media. The peptide is active against bacteria, fungi and promastigotes of *Leishmania major* (dos Santos Cabrera et al., 2004; Rangel et al., 2011). It also induces hemolysis in human erythrocytes, the release of granules and histamine from several vertebrate mast cell types, and blocks the lobster neuromuscular transmission at the level of the postsynaptic membrane (Konno et al., 2000). This wide spectrum of activities indicates that EMP-AF is a rather non-specific cytotoxic molecule and that *A.flavomarginatum* micado certainly cumulates the benefits from the high ability of EMP-AF to disrupt a variety of cell membranes. But what could be the primary role of EMP-AF and under which constraints could its selection have been driven? One of the original aspects of the species is that the female provides an important amount of food to each one of the eggs it lays in the ceiling of a pre-existing cavity in trees, bamboos and reed tubes (Itino, 1986; Okabe and Makino, 2003). Each egg is provided with larvae of pyralid or tortricid moths and then isolated from other eggs by a partition with mud made by the female wasp. The primary role of the venom and perhaps that of EMP-AF in particular, would thus be to quickly and durably paralyze prey and to ensure their long lasting preservation from microbial development to safeguard the nutritional resources of the developing larvae. Possibly, it may also help to control the disease transmission risk inherent to the presence in some nests of *A.flavomarginatum* micado of the cleptoparasitic mite, *Kurosaia jiju*. Okabe and Makino (2003) have demonstrated that tritonymph and adult mites feed on hemolymph of paralyzed caterpillars and occasionally on wasp larvae, whereas mite larvae and protonymphs scavenge organic debris. Should the antimicrobial properties of EMP-AF be effective enough in envenomated prey, it may avoid horizontal transmission of pathogens between prey, mites and wasp larvae. The fact that experiments led in semi-natural conditions revealed no negative impact of *K. jiju* on the survival and development of the wasp's progeny could support this hypothesis.

Three cytolytic peptides (OdVP1, OdVP2 and OdVP3) active against fungi and weakly active against bacteria have been isolated from the venom of the subsocial potter wasp *Orancistrocerus drewseni* (Murata et al., 2009; Baek and Lee, 2010; Baek et al., 2011). Despite low peptidic sequence conservation, OdVP1 (initially named eumenine mastoparan-OD) and OdVP3 also have secondary structure features similar to mastoparans isolated from social Vespinae and Polistinae species. OdPV2 (orancis-protonectin) is a dodecapeptide with sequence similarity to protonectins (hemolytic venom peptides) isolated from several social wasps. *O. drewseni* lives as a subsocial predator of caterpillars (mainly Pyralidae) that are paralyzed for a long time but not killed upon venom injection. As in the previous case, paralyzed prey are transferred into the nest of *O. drewseni* to serve as food for the wasp's progeny. Baek and Lee (2010) hypothesized that the strong antifungal activities of OdVP1–3 could contribute to the preservation of prey once injected but as for EMP-AF, this hypothesis has not yet been validated experimentally. On the other hand, artificial injections of OdVP1–3 into the head and thorax of *Spodoptera exigua* larvae induced a transient paralysis followed by a definitive feeding disorder that lasted until injected larvae died of starvation (Baek et al., 2011). These results indicate that OdVPs may also act as non-specific neurotoxins or as myotoxins. The more potent activity of these peptides against the zwitterionic cell membrane of eukaryotes than against the anionic membrane of prokaryotes may account for their relative versatility, similarly to EMP-AF. Comparable results were obtained with four other cationic linear α-helical peptides from the eumenine wasp, *Eumenes pomiformis* (Baek et al., 2011). Several additional venom AMPs were characterized from other Eumenidae species and from some solitary species belonging to Pompilidae, Apidae and Megachilidae (Table 2). Their

exact biological roles, and notably their potential to regulate infections in animals they sting are however still unclear.

Anoplin is probably one of the shortest venom AMPs characterized to date. It was isolated from the venom of the spider wasp *Anoplius samariensis* (Pompilidae) and displays sequence similarity to the venom AMP crabrolin from the European hornet *Vespa crabo* (Krishnakumari and Nagaraj, 1997; Konno et al., 2001). It may contribute to prey conservation but this has not yet been demonstrated.

The cuckoo bee *Melecta albifrons* (Apidae) is a cleptoparasitic bee that enters the nest of pollen collecting bees and lays its eggs in nest cells of the host species. When the progeny of *M. albifrons* hatches, cuckoo bee larvae not only consume the host larvae's pollen supply but also kill and eat the developing resident larvae (Čeřovský et al., 2008). The major component of *M. albifrons* venom is melectin, an 18 amino acid residue long peptide exhibiting both antibacterial and mast cell degranulating (MCD) activity and low hemolytic activity. The structure of melectin differs from other venom cationic peptides and it shows no homology with known mastoparans. According to the authors, the primary role of melectin would be to protect the solitary female against enemies, while its antimicrobial activity would be more or less secondary. However, melectin was recently found to be HIV-inhibitory (Wang et al., 2010), a property also displayed by ponericin L2 from venom of the ant *Pachycondyla goeldii* and previously demonstrated for honeybee venom melittin (Wachinger et al., 1998).

Another antimicrobial peptide with weak hemolytic activity has recently been found in the solitary red mason bee *Osmia rufa* (Apoidea, Megachilidae) which nests in burrows or crevices in dead wood, clay walls and soil (Stöcklin et al., 2010). This peptide, named osmin, corresponds to the main peptidic product found in the venom. Its C-terminal part contains four residues identical to the C-terminal residues of bombolitins 1–4 from *Megabombus pennsylvanicus* (Argiolas and Pisano, 1985), suggesting that it may be related to members of the MCD peptide family found in Bombinae. Although the venom of *O. rufa* mainly serves for self-defence, it has been proposed that it is also used for protecting the nest from bacterial or fungal infections. Again, this hypothesis has not yet been tested experimentally.

These examples show that several solitary and subsocial wasps have evolved versatile cytolytic molecules possessing significant antimicrobial properties. They appear thus to have a biochemical venom arsenal theoretically able to limit infections in stung animals. However further experimental works are required to confirm their biological role(s) in natural conditions. An interesting point is that among venom AMPs identified from Eumenidae species many, but not all, share similar structural features and biological activity with peptides from mastoparan family, widely distributed in social Vespidae and Polystinae (Nakajima, 1986). This situation mirrors that of Apoidea in which similar venom components, unrelated to mastoparans but equally exhibiting MCD and antimicrobial activities were found in the solitary Megachilidae *O. rufa* and the social species *M. pennsylvanicus*. A noticeable difference here resides in the greater primary sequence similarity between these compounds that leaves open the possibility of the conservation of some ancestral antimicrobial venom components within Apoidea. Interestingly venom antimicrobial components were also described from ants that use their venoms for predation and/or defense. It indicates that although predatory and social lifestyles imposed the increased use of venoms for defensive and offensive purposes, a background antimicrobial function has been conserved or convergently recruited in these venoms, possibly because of a high pathogen pressure exerting on all these species. Up to now, the importance of microbial pressures in shaping the evolution of the composition of these hymenopteran venoms has almost never been evaluated and taken into consideration.

3.3.3. Ants

Worker fire ants use their venom as an offensive weapon towards sympatric arthropod and vertebrate competitors and predators (Schmidt, 1986c). Their sting is lethal to arthropods and small vertebrates (Fitzgerald and Flood, 2006). In large mammals, 50–100 simultaneous stings are sufficient to induce systemic toxic reactions (Fitzgerald and Flood, 2006). However, in most normal cases (i.e. in which no hypersensitive reactions occur), envenomations by red (*S. invicta*) and black (*S. richteri*) imported fire ants induce a local wheal-and-flare reaction followed by immediate pain, inflammation and intense pruritus. Venom alkaloids cause the formation of a papule (cellulitis reaction) which evolves into a sterile pustule, cleared from bacteria, at the site of the sting (Fitzgerald and Flood, 2006; Chen et al., 2009). Within 24 h, the pustule appears to be filled with necrotic tissue with cellular infiltration of lymphocytes, eosinophils, and polymorphonuclear cells. In addition to their cytolytic activity, fire ants venom alkaloids exhibit hemolytic, antibacterial, algogenic and insecticidal properties. Three venom alkaloids from *S. invicta* (solenopsin A, B and C) have been shown to possess a direct antimicrobial activity against Gram-positive bacteria, and to a lesser extent, against Gram-negative bacteria (Jouvenaz et al., 1972) which likely directly contributes to the pustule asepsis. The pustule usually heals and resolves spontaneously in the following weeks. Nevertheless, Fitzgerald and Flood (2006) mentioned that in companion animals pustules caused by fire ants can superinfect if the epidermal covering is scratched off. Secondary bacterial infection can then establish itself and potentially become systemic. Note however that all *Solenopsis* species do not share the same ability to induce sterile pustules. For instance, *S. geminata*, *S. xyloni* and *S. aurea* do not induce pustule in human skin (Schmidt, 1986c). On the other hand, the application of venom on the body surface as a way of protecting against pathogens has been suggested for the ant *S. invicta* by Obin and Vander Meer (1985).

Two antimicrobial peptides, pilosulin 3 and 4, have been characterized by Inagaki et al. (2004) from the venom of the Australian jumper ant *M. pilosula*. Both peptides display low sequence similarity to melittin and exhibit antibacterial activity towards Gram-negative and Gram-positive bacteria. They would contribute to sterilization of captured prey. Both peptides are acknowledged allergens (IUIS Allergen Nomenclature, 2012). The venom of this species also contains another major allergen, myr p 1 (also named pilosulin 1) which has been shown to possess strong cytotoxic and hemolytic activities (Wu et al., 1998).

Most Ponerinae predators use their venom to capture their prey, which are immediately brought back to the nest by solitary foraging workers. Orivel et al. (2001) have suggested that the presence of at least 15 different antimicrobial peptides in the venom of the arboreal Ponerinae *P. goeldii*, could contribute to avoid colony contamination by microbes infecting stung prey. These venom AMPs were clustered into three groups, named ponericin G (seven peptides), W (six peptides) and L (two peptides), according to their primary structure similarities. The biological activities of ponericins G vary greatly despite a high level of intra-group sequence conservation. Ponericins G1 and G3 are active against Gram-negative and Gram-positive bacteria, fungi and yeasts. They are also insecticidal but not hemolytic. These features suggest a relative specificity toward eukaryotic cells. A representative member of this group, ponericin G1, shares 60% sequence similarity with cecropin, an inducible AMP almost exclusively produced by lepidopteran and dipteran species. With exception of W6, ponericins W exhibit antimicrobial, hemolytic and insecticidal actions. Intriguingly, W1 and W3 share 70% sequence similarity with honeybee melittin and strong amino acid sequence similarity with two ponericin-like antimicrobial peptides (Plamp), recently isolated from the venom of the Chinese swimming scorpion *Lychas mucronatus* (Ruiming

et al., 2010). Ponericin L2 was found to be active against bacteria but not against fungi. It also possesses an inhibitory action towards HIV-1 in permissive culture cells (Wang et al., 2010). Recently, six venom peptides from the giant neotropical hunting ant *Dinoponera australis* referred as Dinoponeratoxins have been isolated and their sequences compared to sequences of other known venom components (Johnson et al., 2010). This work allowed the identification of two ponericin G-like peptides (Da-3105 and Da-3177) that showed significant sequence similarity with ponericin G2 and G3. Two additional peptides (Da-2502 and Da-1837) where found similar to ponericin W3 and to the antimicrobial peptide mucroporin, originating from the scorpion *L. mucronatus*, respectively. The evolutionary implications of these important finding are worthy of further investigation in order to elucidate the origins and functional evolution of antimicrobial arsenals that are present in venoms from distant arthropod species.

3.3.4. Social Apidae and Vespidae

In non-allergic mammals, stings of social Apidae and Vespidae induce toxic reactions characterized by onset of pain, erythema, oedema and pruritus (Vetter et al., 1999; Fitzgerald and Flood, 2006). Toxic reactions and pain are caused by the injection of hemolytic, vasoactive, myotoxic or neurotoxic venom compounds acting alone or synergistically. These molecules belong to different biochemical classes including biogenic amines, peptides and enzymatically active proteins (Argiolas and Pisano, 1985; Banks and Shipolini, 1986; Nakajima, 1986; de lima and Brochetto-Braga, 2003; Chen and Lariviere, 2010). Several venom constituents display a lowly selective membrane-disrupting potential conferring them multifunctional properties encompassing antimicrobial and defensive functions. Honeybee melittin and mastoparans from social wasps and hornets figure among the best-studied versatile venom components.

Melittin, an amphipathic 26-amino acid peptide which accounts for 30–50% of the dry weight of honeybee venom possesses antimicrobial, antiviral, nociceptive, MCD and hemolytic activities due to its ability to interact with both layers of biological membranes (Ownby et al., 1997; Bernèche et al., 1998; Wachinger et al., 1998; Chen and Lariviere, 2010; Nishikawa and Kitani, 2011). It also enhances the activity of other venom constituents, such as PLA2 (Banks and Shipolini, 1986), activates the victim's endogenous PLA2 activities (Argiolas and Pisano, 1983) and constitutes one of the 12 officially recognized venom allergens from *A. mellifera* (IUIS Allergen Nomenclature, 2012). At first described from *Vespula lewisii* (Hirai et al., 1979), mastoparans from social wasps (found since in *Agelaia*, *Vespula*, *Protonectarina*, *Protopolybia*, *Parapolybia*, *Polybia* and *Polistes* genera) and hornets (*Vespa* genus) form a family of structurally similar peptides that also display antimicrobial, hemolytic and MCD activities and can facilitate endogenous PLA2 activity (Argiolas and Pisano, 1983; Dohtsu et al., 1993; Mendes et al., 2005; de Souza et al., 2011). Biochemical and structural features of other venom AMPs from wild eusocial bees, bumblebees, social wasps and hornets are summarized elsewhere (de lima and Brochetto-Braga, 2003; Kuhn-Nentwig, 2003; Slaninová et al., 2012) and it did not appear necessary to reproduce this information here.

Abscess, impetigo, ecthyma or cellulitis are various manifestations of cutaneous secondary infections, observed in mammals, and frequently associated with insect, spider and snake bites and envenomations by numerous organisms (Hubbard and James, 2011; Krecsák et al., 2011). Thanks to Veraldi et al. (2011), inquiring minds will be able to contemplate, for instance, a superb abscess on a right buttock upon envenomation by a *Conus* gastropod. In contrast, secondary infections seem to be much more rarely associated with single envenomations by social vespids and bees, at least in human and companion animals (Fitzgerald and Flood, 2006). In human the few known cases of life-threatening secondary infections that followed bee or vespid stings occurred in hyper-reactive or hypersensitive patients.

Anderson et al. (1995) have reported the triggering of a Well's syndrome (characterized by extended eosinophilic cellulitis and triggered by a variety of stimuli) in a female child following a honeybee sting. Septicaemia followed but only several weeks after envenomation. This child did not display IgE-mediated hypersensitivity but could have suffered another form of venom hypersensitivity (e.g. non allergic hypersensitivity or IgG-mediated allergy). In another work, Koçer et al. (2003) reported two exceptional cases of skin and local soft tissue necrosis after Hymenoptera (unidentified hornet and yellow-jacket) stings. These rare reactions were attributed to a delayed hypersensitive reaction and were followed by superinfection of the necrotic areas in the 2 weeks after envenomation. In each of these cases, secondary infections occurred in hypersensitive patients several weeks after the initial sting and involved species commonly present on human skin (e.g. *Staphylococcus aureus*). The important skin lesions induced by venom hypersensitivity in all these patients most probably favored secondary infections. Such cases remain so exceptional that these types of reactions were not included in the classification systems of reactions to hymenopteran stings at the date of submission of the most recent study (Koçer et al., 2003).

In most normal cases, the sting sites are very likely sterilized by the immediately induced, short-term local inflammatory reaction. Most probably they are also sterilized by the action of the above mentioned venom antimicrobial molecules, which can spread and diffuse into the envenomated tissues for up to 2 h (Francese et al., 2009). Once cleared from pathogens, the autotomized sting that would not have been removed can thus remain inert in the body of stung individuals up to several decades, even in vulnerable organs. For instance, Gilboa et al. (1977) have reported an extreme case in which a wasp sting to the upper right eyelid of a patient caused an initial painful edema which spontaneously regressed few days later. Unbeknownst to the patient, the wasp aculeus has then been retained for 28 years in its right eye without causing inflammation. A similar situation was observed in another patient, in which a bee sting has been retained in the cornea for 21 years with no signs of inflammatory activity.

Another more subtle mechanism, in relation to venom-induced pain, can be proposed to explain the low prevalence of secondary infections occurring in human patients or large mammals after bee and social wasp stings. Venom-induced pain is traditionally interpreted as a negative stimulus imprinting avoidance behavior on a predator (Schmidt, 1986c). On the other hand, itching is commonly reported following hymenopteran stings and is a main cause of occasional secondary infections occurring for instance upon fire ant stings (see above). Interestingly, inhibition of itching by painful stimuli has been experimentally demonstrated in mammals by use of various painful thermal, mechanical, and chemical stimuli (Schmelz, 2005), although it is now acknowledged that these sensations are mediated by distinct neural circuits (Sun et al., 2009). One can speculate that venom algogenic components not only have a deterrent effect on large animals and may act as honest signals indicative of on-going tissue damages, but may also temporarily moderate its itching behavioral response. The time lapse during which itching would be attenuated could allow venom antimicrobial components and endogenous molecules liberated by the inflammatory process to eliminate residual pathogens within envenomated tissues, hence contributing to a relative asepsis at the sting site.

An intriguing question needs then to be addressed: to what extent could the venom-mediated "cleaning effect" observed here be advantageous for stingers using their venom as a defensive weapon? Despite their complexity and versatility, venoms of social

Hymenoptera are moderately toxic to vertebrates (Schmidt, 1986c). A noticeable exception in Hymenoptera concerns the venoms of 15 ant species of the genus *Pogonomyrmex* (Myrmicinae) which would be as lethal to mice (0.10–0.62 mg/kg by intraperitoneal injections or Ip) (Schmidt, 1986c) as the venom of the Russell's viper (*Daboia russelii*, 0.4 mg/kg by Ip), one of the most dangerous Asian snake (Engelmann and Obst, 1981; Kumar and Gowda, 2006). If threshold values for lethality are far to be reached by individual venom injections, the force of social species is evidently to inflict, through the recruitment of hundreds of individuals, a massive envenomation (Michener, 1975). Hence, in an extreme case of fatal mass attack by Africanized honeybees (*A. mellifera scutellata*), the whole venom quantity still circulating in the serum of the deceased patient 1 hour after the attack was estimated at 27 mg (França et al., 1994). This huge quantity represents the equivalent of the overall venom protein content of more than 350 venom sacs from *A. mellifera* or of approximately 20–30,000 *Cotesia congregata* parasitoid females. Even in non-allergic people, massive envenomations may cause systemic reactions, renal failure or cardiac arrest due to consequences of the acute venom toxicity (Vetter et al., 1999). Our own LD_{50} for honeybee stings without appropriate medical treatment would correspond to 500–1200 stings (Vetter et al., 1999; Fitzgerald and Flood, 2006). During their collective assaults, winged social Hymenoptera generally concentrate on vulnerable parts (e.g. head, hands and feet during attacks by *Vespa velutina*) (de Haro et al., 2010; E. Darrouzet and J. Gevar, personal communication). Successive stings may thus occur on same or very close skin areas. In the absence of sting site sterilization, the risk for horizontal transfer of pathogens from the stung animal to its aggressors and also potentially between stingers could be high. Sterilizing multiple and overlapping sting sites may be advantageous for social Hymenoptera involved in mass attacks: the more the colony would have to invest members in an attack against a large opponent, the safer this action would become with respect to risks of disease importation into the colony. Note that bees of the genus *Apis* have evolved a more radical option that overcomes the risk of colony contamination: the almost systematic autotomy of their sting apparatus observed upon envenomation of vertebrates (Mulfinger et al., 1992). Since venom is apparently sprayed by foragers on the cuticle of other members of the colony (Baracchi et al., 2011), avoidance of sting apparatus contamination could be crucial for social species.

4. Conclusion

In stinging Hymenoptera, as in other venomous animals, the composition and functions of the venom is particularly well adapted to face major constraints affecting their main life traits: manipulation of host physiology, capture of prey, defense against competitors and predators and for some ant species, inter-individual communication. In consequence the adaptive significance of the variety of roles fulfilled by hymenopteran venoms has almost always been interpreted with respects to interactions between these insects and other eukaryotic macroorganisms. This main stream conception has long led to neglect the fundamental role that venoms also play in interactions with pathogenic, parasitic, commensal or mutualistic microorganisms. Yet, these organisms also exert high selective pressures on Hymenoptera. Whatever their life styles, numerous evidence points out that many hymenopteran species have evolved venom features that actively participate in the regulation of microbial infections, both in stinging and stung individuals.

Despite tens of millions of stings occurring annually, no significant infectious disease that could be vectored by Hymenoptera of medical importance seems to have been described so far. Considering the propensity of microbial pathogens and parasites to use insects as vectors, this observation is rather surprising. It may partly be explained by the apparent effectiveness of individual and collective defenses and prophylactic means these insects have evolved against infectious organisms. In addition it has recently been shown that venoms of some species can play a significant role in these processes in contributing to prevention of disease transmission in a social immunity context. Other putative elements related to characteristics of the stinging activity of social Hymenoptera and their venom composition could also contribute to the apparent absence of transmission of pathogenic agents to humans through accidental stings.

At the organismic level, the intrinsic features of hymenopteran venoms and venom apparatuses appear globally hostile to successful infections by microorganisms with the exception of some viruses and particular fungi. Microbial diffusions into these organs and from here to the rest of the body are possibly limited by biochemical and physical barriers depending on the venom composition and on the histologic organization of the venom apparatus. In consequence, few pathogens seem to be able to use venom producing organs as a natural route of infection and even fewer have apparently succeeded in establishing symbiosis in the venom apparatus of known actual species, during the course of evolution. Many venoms could play a key role in controlling the risk of secondary infections in stung hosts, prey and even predators, either directly, thanks to more or less versatile antimicrobial molecules they contain, or indirectly *via* the selective preservation or stimulation of endogenous antimicrobial defenses in stung individuals.

Antimicrobial properties of hymenopteran venoms have recently gained in interest in order to identify and optimize natural antimicrobial products able to supplement classical therapeutic agents against multi-drug resistant pathogens. This crucial need has led to the description of an increasing number of venom components, notably peptides, displaying antiviral, antibacterial, antifungal, and/or anti-parasitic activities. Besides the useful characterization of biological activities, crucial future works are also required to confirm with natural pathogens and *in vivo* the biological and ecological significance of venom antimicrobial strategies. A careful search for microorganisms having succeeded in circumventing such strategies and in establishing within venom apparatuses could reveal a lot of information. At an evolutionary level, another future challenge will be to explore relevant evolutionary scenarios explaining the presence of some peptides and proteins sharing high amino acid sequence similarity and functional conservation in venoms of unrelated venomous species (e.g. Hymenoptera and scorpions). It leaves exciting challenges to next generations of entomologists and investigators in insect sciences.

But at the moment, they are too busy rolling in parks' lush grass.

Acknowledgments

This paper is dedicated in memory of Prof. Nancy Beckage, whose invaluable contribution to the field of parasitoid wasps' physiology has greatly inspired my own works. Her warm support will be missed. Also I wish to thank Germain Chevignon, Axel Touchard, Gabriel Gemmier and my other colleagues for their support and helpful contributions. Many thanks also to my family which is now really fed up with hymenopteran venoms. I hope it has not too much bored you too!

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