Parasitoid venoms: from proteins to zombies to medicine

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Abstract: Parasitoid wasps are insects which require another arthropod host for their larvae to develop. Parasitoid females often inject venoms into the host at the time of oviposition to aid in regulating the host for their larvae. The enormous diversity of parasitoids has led to a great diversity in their venoms as well, but few venoms have actually been characterized in function or composition. Known venom cocktails typically perform one of two functions depending on the type of parasitoid using them. In ectoparastioids, where the parasitoid develops on the outside of the host, venoms are often used for paralyzing the host whereas endoparasitoids, which develop inside the host, use the venoms primarily for regulation of the host, often controlling hormone levels and immune response. Yet even among these groups there is considerable variation in the composition and function of venoms even among parasitoids attacking the same host. The components which are primarily active in the host are typically proteinaceous and have thus received the greatest attention in examining the structure and function of venoms. However, many other venom components, even among well studied species, are still to be examined. Parasitoids are of considerable importance to biological control of arthropod pests and as such, understanding their venoms could be key in their implementation for pest control, especially given the variation among venoms of individuals even within a population of parasitoids. Some research is even currently underway to examine the use of venoms directly for pest control. Additional research could also advance the medical field, particularly with the discovery of venom components that could be developed into new pharmaceutical drugs. This review will examine the current knowledge of parasitoid venoms focusing on their chemical composition, function in relation to their hosts, and the implications of parasitoid venom research.

Introduction

Female insects belonging to the order Hymenoptera are capable of producing venoms which can be injected using their modified ovipositors (Poirié et al. 2014). Within this group, is a great diversity of parasitoids, insects which require another arthropod host to complete their larval development, which may account for as much as 20% of the animals on the planet (Godfray 1994). Their diversity and obligatory parasitic lifestyle has led parasitoids to develop an astounding array of methods for manipulating their host to the benefit of their larvae. Upon oviposition, female parasitoids inject a complex of calyx/ovarian fluid, polydnaviruses, teratocytes, and/or venoms into their host to create a more favorable environment for their progeny (Beckage and Gelman 2004, Moreau and Asgari 2015). The functions of these maternal factors vary widely among parasitoids and are often very specific to the host and the life history of the parasitoid larvae (Beckage and Gelman 2004). Not every species produces all of these factors in their oviposition "cocktail", but all parasitoid wasps use some form of venom (Moreau and Asgari 2015).

Traditional definitions of venoms describe them as a paralytic or fatal toxin, but more recent definitions have expanded to include any secretion injected through a wound that causes physiological changes in the host to support the venom producer (Asgari and Rivers 2011). Like the venoms of many other animals in the animal kingdom, parasitoid venoms are a mix of both proteins and non-protein substances (Moreau and Asgari 2015). These proteins are usually secreted (Asgari and Rivers 2011), but venom components in some parasitoids such as aspartylglucosaminidase (AGA), which helps cleave glycoprotein bonds, may aid in secretion by modifying protein structures rather than being secreted themselves (Poirié et al. 2014). Other

components may aid in stabilization or self-protection from venoms. Protease inhibitors (specifically phenyloxidase inhibitors) in *Pimpla hypochondriaca* aid in preventing damage to tissues in the venom sac (Asgari and Rivers 2011). Many venom proteins are likely derived from other functions within the parasitoid. This argument is strengthened by the presence of proteins in some wasps which require only a single peptide change from their normal function to allow secretion. Specific components of venoms differ greatly between species, especially more closely related species parasitizing a common host. Additionally, concentrations of individual components vary due to the differing amounts necessary for bioactivity (Poirié et al. 2014).

Venoms in parasitoids are produced in the posterior abdomen of the wasps so that they can be passed through the ovipositor/stinger (Alves et al. 2015). Venoms are secreted from the venom glands, which appear as long tentacle-like projections around the venom reservoir (Arakawa et al. 2013, Alves et al. 2015). Secretory cells in these glands often have a large nucleus, secretory apparatus, and enlarged endoplasmic reticulum (Arakawa et al. 2013). Beyond this, there is likely a lot of variation in structure of venom apparatuses for which information is lacking, but the small size of parasitoids makes further examination of venom apparatus anatomy difficult (Alves et al. 2015).

Parasitoid venoms function differently based on the two major categories of parasitoid lifestyles: ectoparasitoids and endoparasitoids (Asgari and Rivers 2011). Ectoparasitoid larvae develop on the exterior of the host and must therefore find a way to immobilize the host and prevent it from molting so that the larva does not become dislodged. This lends ectoparasitoids to most often be idiobionts, parasitoids which arrest host development. On the contrary,

endoparasitoids tend to be koinobiont, allowing the host to continue development as the larval parasitoid grows. Koinobiont endoparasitoids have their own set of difficulties, however, and are forced to deal with the immune system and ever changing physiology of their host. Venoms of parasitoids tend to reflect these life history differences (Asgari and Rivers 2011). Koinobiont ectoparasitoids have been documented (ex. MacDonald and Caveney 2004, Takasuka and Matsumoto 2011, Souza et al. 2015) as well as some cases of idiobiont endoparasitoids (Tena et al. 2008, Mabiala-Moundoungou et al. 2010) but few of their venoms have been extensively studied.

Venoms of parasitoids are overall a rather understudied subject (Moreau and Asgari 2015). Those venoms that are known in detail are primarily from just a handful of well-studied model parasitoids including the aphid parasitoid *Aphidius ervi* (Colinet et al. 2014), and *Nasonia vitripennis* (Martinson et al. 2014). This review will address the current knowledge of the structure and function of parasitoid venoms as well as some potential implications of venom research and some future directions for venom studies.

Ectoparasitoid venoms

Ectoparastioid venoms are often involved in causing paralysis and preventing development arrest of the host insect (Moreau and Asgari 2015). However, even within this subgroup of parasitoids, there is great diversity among the venoms. Many ectoparasitoid venoms impact the endocrine system of the host to change its development so that it is most advantageous to the parasitoid, such as inhibiting molting to prevent the parasitoid from being shed from the host (Beckage and Gelman 2004). One such example is found in *Euplectrus spp.*, where the venom inhibits apolysis and ecdysis. Interestingly, the venom prevents apolysis if

injected prior to molting, and prevents ecdysis if apolysis has already occurred. Other ectoparasitods may inject potent paralyzing toxins to prevent host development, such as *Bracon hebetor* whose venom causes permanent paralysis at doses of only .005 ppm (Beckage and Gelman 2004). Venoms of the few koinobiont ectoparasitoids have not been explicitly examined, but at least in some transient paralysis is induced at oviposition (Souza et al. 2015) and may be caused by venom. Koinobiont ectoparasitoid venoms should be further examined for comparison of their effects in relation to other koinobiont endoparasitoid venoms.

One of the more well studied ectoparasitoid venoms is that of *Nasonia vitripennis*, a parasitoid of flesh flies, likely because it is a genetic model with a fully sequenced genome (Martinson et al. 2014) and because it does not inject polydnaviruses with its venom as many other parasitoids do (Danneels et al. 2014). Approximately 79 venom proteins have been identified from this parasitoid, including some with structures similar to odor-binding proteins or antimicrobial peptides (Qian et al. 2013). Venom of *N. vitripennis* is very quick acting, causing death of plasmatocytes and inhibiting the activity and spread of other hemocytes within only an hour of envenomation (Beckage and Gelman 2004). In addition to this immune suppression, *N. vitripennis* decreases respiration and pyruvate metabolism in its pupal host (Martinson et al. 2014). This parasitoid's venom has actually been shown to cause complex metabolic cascades in pupae of *Sarcophaga bulleta*. Overall, amino acid production and sorbitol metabolism were increased by envenomation, while glycolysis was down regulated and chitin biosynthesis was inhibited (Mrinalini et al. 2015).

Another study by Danneels et al. (2014) also demonstrated that some parasitoids can be bioactive in organisms other than the target host. Venom from *N. vitripennis* caused an anti-

inflammatory response in mammalian cells. The venom inhibits NF-κB transcription factors which regulate expression of a variety of inflammation and immunity related genes. The venom is also cytotoxic at higher doses but the anti-inflammatory action could be achieved at lower concentrations. Interestingly, the venom interacts by different mechanisms in mammalian cells than in insects, which could warrant further investigation on bioactivity of venoms (Danneels et al. 2014).

Catolaccus grandis, which attacks cotton boll weevil larvae, was able to manipulate host physiology simply by surface contact with the host larva (Morales-Ramos et al. 1995). This is not necessarily surprising since females probe the host to ensure it is suitable but actually lay the egg nearby the host rather than on or in it. Topical application of venom was shown to cause high mortality in the weevil larvae. Additionally, hosts that did survive showed reduced feeding and higher doses of venom cause immediate paralysis (Morales-Ramos et al. 1995).

One parasitoid that has a particularly unique venom is the jewel wasp *Ampulex compressa*, which parasitizes cockroaches. Attack on the cockroach by the jewel wasp involves two separate envenomation events. First, the host is stung in the thorax inducing a one to two-minute transient paralysis to the cockroaches' legs which allows a second sting directly into the central nervous system of the cockroach's head (Libersat and Gal 2014). This second sting causes the cockroach to groom itself excessively through injection of dopamine in the venom of the parasitoid. This effect lasts for approximately 30 minutes which is nearly the exact time it takes the parasitoid to seek out a nest to provision with its cockroach prey (Weisel-Eichler et al. 1999).

After the wasp returns, the more interesting effect of the venom kicks in and the cockroach effectively becomes a zombie (Libersat and Gal 2014). At this point the cockroach goes into a hypokinetic state in which it becomes incapable of initiating an escape response in particular (Gavra and Libersat 2011). The cockroach appears to lose the will to escape so that changes in air pressure on the cerci and contact with the antennae both fail to provoke the cockroach to escape as they would normally (Libersat and Gal 2014). Many details of the mechanisms surrounding this peculiar venom induced behavior are unclear (Libersat and Gal 2014). Opioid receptors likely play a role (Gavra and Libersat 2011), and artificially depleting monoamines in the central nervous system causes a similar effect, but venom itself does not deplete amines or inhibit monoamine production (Banks and Adams 2012). This hypokinesia even causes cockroaches to rest and float more when placed in water where they would normally swim frantically. However, the effect does eventually wear off after 3-7 days, after which time the larval parasitoid is well into feeding on the helpless cockroach (Libersat and Gal 2014).

Endoparasitoid venoms

The number of venom components known from endoparasitoids seems to outweigh the small number of known ectoparasitoid venoms (Moreau and Asgari 2015). Because of their generally koinobiont life strategy, endoparasitoids do not usually employ permanent paralyzing effects in their venom (Asgari and Rivers 2011). Rather, endoparasitoid venoms are more often geared towards control of the host immune system and physiology to facilitate larval development (Moreau and Asgari 2015). Endoparasitoids also often inject polydnaviruses

and/or teratocytes with their venom which often synergize to further influence the host physiology (Beckage and Gelman 2004).

However, there are often exceptions to the norm in nature. Effects of parasitism by the few idiobiont endoparasitoids appear more similar to that of ectoparasitoids (Tena et al. 2008, Mabiala-Moundoungou et al. 2010). The potent venom of *Asobara japonica*, a parasitoid of *Drosophila melanogaster* larvae (Mabiala-Moundoungou et al. 2010). Dose dependent envenomation by *A. japonica* causes immediate permanent paralysis in the host or even death. The venom is actually so potent that a component of the ovarian fluid injected by the female parasitoid is necessary to prevent the death of the host. The reason for retention of such a potent venom remains unclear (Mabiala-Moundoungou et al. 2010). Future research could provide explicit comparisons of endoparasitoid and ectoparasitoid idiobiont venoms. If combined with modern genetic study techniques, this could provide insight into the evolution between endo- and ectoparastioids. Many species of the *Asobara* genus share a number of characters with ectoparasitoids, including inducing paralysis and the lack of virus or teratocytes in their oviposition fluids and may be transition species in the evolution between endo- and ectoparasitoids (Beckage and Gelman 2004).

Other members of the *Asobara* genus have also been shown to induce paralysis in their host. Venom of *A. tabida* causes transient paralysis in *Drosophila* hosts and additionally causes host mortality prior to pupation in the absence of a larval endoparasitoid. Factors produced by the larval wasp act to combat the mortality effects of the venom. The major component in *A. tabida* venom, an aspartylglucosaminidase-like protein may be responsible for the transient paralysis by producing the neurotransmitter aspartate (Asgari and Rivers 2011). The purpose of

transient paralytic components in endoparasitoid venoms is unclear, but examination of two *Binodoxys* aphid parasitoids lends support for a role in avoiding multiple oviposition events by a single female (self-superparasitism)(Desneux et al. 2009).

Most other endoparasitoid venoms primarily affect the immune response of the host (Asgari and Rivers 2011). Venom of *Cotesia glomerata* suppress melanization of hemocytes in its imported cabbageworm host, an important component in encapsulation, the primary defense strategy of insects against parasitoid invasion (Kitano 1982). A similar effect was observed by a serine protease homolog from *C. rubecula* venom which interferences with the activation of prophenoloxidase which aids in forming melanin in the hemolymph (Asgari et al. 2003). *Pteromalus puparum*, a pupal endoparastoid of the imported cabbageworm also has venom components which inhibit the cellular immune response and encapsulation ability of its host (Mabiala-Moundoungou et al. 2010).

Two of the largest parasitoid families, the braconids and ichneumonids, employ polydnaviruses (PDVs) in addition to their venom to aid in suppressing host immunity. Venoms in these wasps often synergize with PDVs by aiding in the uptake of virions by host cells and help uncoat the viral DNA to speed up infection by the viruses (Beckage and Gelman 2004). Some parasitoids even have virus-like particles (VLPs) in their venom which have been demonstrated to cause apoptosis in hemocytes in parasitism by *Leptopilina sp.* (Richards et al. 2013) and *Meteorus pulchricornis* (Suzuki and Tanaka 2006). These particles are membrane bound vesicles, likely cellular in origin, that may deliver venom proteins to host cells (Poirié et al. 2014).

Venom from *Pimpla hypochondriaca*, a parasitoid of primarily noctuid moth larvae, was able to reduce the immune ability of its host using its venom and cause greater susceptibility to fungal (Marris et al. 1999) and bacterial infection (Richards et al. 2013). In addition, the volume and periodicity of CO_2 emission from respiration is decreased (Marris et al. 1999). The host range of *P. hypochondriaca* is notably large and may be reflected in its venom components as well. A very large mixture of polypeptides was found in its venom and many of them have similar anti-hemocyte properties (Parkinson et al. 2002).

Arguably the endoparasitoid venom of greatest interest has been that of *Aphidius ervi*, which parasitizes the model pea aphid *Acyrthosiphon pisum* (Colinet et al. 2014). Envenomation by *A. ervi* results in castration of the aphid host through breakdown of germarial cells and inducing apoptosis. This may be a strategy to conserve resources for the parasitoid that would otherwise have been allocated to costly germ cells (Digilio et al. 2000). Venom of *A. ervi* contains γ-glutamyl transpeptidases as the most abundant protein but also includes serine protease homologs and a unique leucine-rich domain protein (Colinet et al. 2014). Interestingly, proteins of *A. ervi* are largely not glycosylated, unlike many other endoparasitoid venom proteins (Digilio et al. 2000). Endoplasmin has also been identified from *A. ervi* venom, which is most likely involved in stabilizing, transporting, and secreting other venom proteins (Colinet et al. 2014). Though the functions of many of the specific components of *A. ervi* venoms have not been identified, the venom alone has a well demonstrated castration effect and is sufficient for developmental arrest and even death of the host (Digilio et al. 2000).

Implications and future directions of venom research

Venoms of parasitoids are poorly studied (Moreau and Asgari 2015), maybe in part due to their small size and thus difficulty of study compared to other venomous animals (Alves et al. 2015). But parasitoids are undoubtedly important in biological control (Asgari and Rivers 2011) and studying their venoms is an important part of understanding their interactions with their host (Alves et al. 2015). A better understanding of parasitoid venoms could lead to improved pest control strategies and fill much needed gaps in pharmaceutical research with their enormous diversity of bioactive compounds.

A number of potential medical applications have already been demonstrated with parasitoid venoms. Venom of *N. vitripennis* has shown anti-inflammatory effects in mammalian cells lines below cytotoxic levels (Danneels et al. 2014). Further research on the individual components of this venom could lead to development of new anti-inflammatory drugs for which there is high demand. Additional research could also focus on the mode of action of these venom components. The differential method of effect between mammalian and insect cells might reveal new information on immune activation pathways (Danneels et al. 2014). Modes of action for many other parasitoid venom proteins, such as castration by *A. ervi,* are also in need of investigation (Digilio et al. 2000).

Many other venoms are likely candidates for a rich diversity of new drug developments (Mrinalini et al. 2015). Serine proteases in venoms demonstrate anticoagulant effects that could be used for treating thrombotic disorders and cytotoxic venoms might be usable as anti-tumor agents . Neurotoxic peptides blocking ion pathways may be valuable in pain alleviation (Moreau and Asgari 2015). Up regulation of the sorbitol pathway, an effect typically observed in hypoglycemia, demonstrated by *N. vitripennis* venom should be investigated for insight in

aiding diabetes research (Mrinalini et al. 2015). Furthermore, venom's natural injectable stability and rapid arrival at target tissues could help in designing enzyme replacement therapies for people with dysfunctional enzyme storage or production (Moreau and Asgari 2015).

A somewhat more obvious impact of parasitoid venom research is improvements in pest control. First, it may be possible to use venom extracts directly as a biopesticide. Alternatively, plant genes could be modified to express venom proteins and, in fact, some patents for similar projects have already been made (Moreau and Asgari 2015). Proteins of some venoms, such as that of *P. hypochondriaca* also increase the susceptibility of insect pests to other pathogens (Marris et al. 1999, Richards et al. 2013). Many large proteins can be delivered by ingestion, so venoms could be applied for ingestion by pests to increase susceptibility to bacteria, or bacteria could be applied which express venom protein genes (Richards et al. 2013). Addition of venom in one study doubled the pest mortality caused by fungal spores (Marris et al. 1999). Various venom chitinases could be used as an active ingredient for pest control as well (Mrinalini et al. 2015).

Furthermore, current control strategies could be improved in addition to the development of novel control strategies. Venom of more generalist parasitoids may contain a large number of proteins and their venom composition may vary based on hosts available. Therefore, examination of venoms could help ensure the proper strain of biological control agent is being implemented (Poirié et al. 2014). Other studies have demonstrated that venom composition does differ between populations (Colinet et al. 2014). Improved expression of venom genes in parasitoids could potentially increase their potency as biological control agents.

Alternatively, venom genes could be expressed by other biological control agents to increase their effectiveness. This would be most applicable to organisms such as *Bacillus thuringiensis*, but care would need to be taken to prevent expansion of the bacteria's host range (Moreau and Asgari 2015).

Conclusion

Overall, there is a need for more studies on parasitoid venoms. Parasitoids are one of the most diverse groups of animals on the planet and their enormous range of hosts lends them to having one of the most diverse chemical toolbox of the animal kingdom. There is a great deal of untapped potential for discovery in this pool of bioactive compounds and venoms are likely a rich source of pharmaceutically relevant compounds. Beyond that, parasitoids remain invaluable in biological control and effective application of them as biological control agents involves a thorough understanding of the mechanisms mediating their interactions with their hosts. Parasitoids have evolved a number of intricate methods of manipulating physiology and there is no group that has learned the ways of host manipulation better. It is even possible that novel methods of control could also be discovered by studying the interaction of toxic venom proteins on insect physiology.

Literature Cited

Alves, T. J. S., V. Wanderley-Teixeira, Á. A. C. Teixeira, L. C. Alves, B. C. Araújo, E. M. Barros, and F. M. Cunha. **2015**. Morphological and histological characterization of production structures, storage and distribution of venom in the parasitic wasp *Bracon vulgaris*. Toxicon. 108: 104–107.

Arakawa, T., H. Tanaka, J. Ishibashi, R. Fujii-Muramatsu, R. Murakami, and N. Nakashima. **2013**. Venom production, venom gland cell fine structure and complementary DNA cloning of a novel cysteine-rich secretory venom protein of the parasitoid wasp *Cotesia glomerata* (L.) (Hymenoptera: Braconidae). Aust. J. Entomol. 52: 387–392.

Asgari, S., and D. B. Rivers. **2011**. Venom proteins from endoparasitoid wasps and their role in host-parasite interactions. Annu. Rev. Entomol. 56: 313–335.

Asgari, S., G. Zhang, R. Zareie, and O. Schmidt. **2003**. A serine proteinase homolog venom protein from an endoparasitoid wasp inhibits melanization of the host hemolymph. Insect Biochem. Mol. Biol. 33: 1017–1024.

Banks, C. N., and M. E. Adams. **2012**. Biogenic amines in the nervous system of the cockroach, *Periplaneta americana* following envenomation by the jewel wasp, *Ampulex compressa*. Toxicon. 59: 320–328.

Beckage, N. E., and D. B. Gelman. **2004**. Wasp parasitoid disruption of host development: implications for new biologically based strategies for insect control. Annu. Rev. Entomol. 49: 299–330.

Colinet, D., C. Anselme, E. Deleury, D. Mancini, J. Poulain, C. Azéma-Dossat, M. Belghazi, S. Tares, F. Pennacchio, M. Poirié, and J.-L. Gatti. **2014**. Identification of the main venom protein components of *Aphidius ervi*, a parasitoid wasp of the aphid model *Acyrthosiphon pisum*. BMC Genomics. 15: 342.

Danneels, E. L., S. Gerlo, K. Heyninck, K. Van Craenenbroeck, K. De Bosscher, G. Haegeman, and D. C. De Graaf. **2014**. How the venom from the ectoparasitoid wasp *Nasonia vitripennis* exhibits anti-inflammatory properties on mammalian cell lines. PLoS One. 9: 1–13.

Desneux, N., R. J. Barta, C. J. Delebecque, and G. E. Heimpel. **2009**. Transient host paralysis as a means of reducing self-superparasitism in koinobiont endoparasitoids. J. Insect Physiol. 55: 321–327.

Digilio, M. C., N. Isidoro, E. Tremblay, and F. Pennacchio. **2000**. Host castration by *Aphidius ervi* venom proteins. J. Insect Physiol. 46: 1041–1050.

Gavra, T., and F. Libersat. **2011**. Involvement of the opioid system in the hypokinetic state induced in cockroaches by a parasitoid wasp. J. Comp. Physiol. A Neuroethol. Sensory, Neural, Behav. Physiol. 197: 279–291.

Godfray, H. C. J. 1994. Parasitoids: Behavioral and Evolutionary Ecology.

Kitano, H. 1982. Effect of the venom of the gregarious parasitoid *Apanteles glomeratus* on its hemocytic encapsulation by the host, *Pieris*. J. Invertebr. Pathol. 40: 61–67.

Libersat, F., and R. Gal. **2014**. Wasp voodoo rituals, venom-cocktails, and the zombification of cockroach hosts. Integr. Comp. Biol. 54: 129–142.

Mabiala-Moundoungou, A. D. N., G. Doury, P. Eslin, A. Cherqui, and G. Prévost. **2010**. Deadly venom of *Asobara japonica* parasitoid needs ovarian antidote to regulate host physiology. J. Insect Physiol. 56: 35–41.

MacDonald, K. E., and S. Caveney. **2004**. Unusual life history characteristics of *Elachertus scutellatus* Howard (Hymenoptera: Eulophidae), a koinobionic ectoparasitoid. Environ. Entomol. 33: 227–233.

Marris, G. C., H. a. Bell, J. M. Naylor, and J. P. Edwards. **1999**. The role of *Pimpla hypochondriaca* venom in the suppression of pupal Noctuid host immunity. Entomol. Exp. Appl. 93: 289–296.

Martinson, E. O., D. Wheeler, J. Wright, Mrinalini, A. L. Siebert, and J. H. Werren. **2014**. *Nasonia vitripennis* venom causes targeted gene expression changes in its fly host. Mol. Ecol. 23: 5918–5930.

Morales-Ramos, J. A., M. G. Rojas, and E. G. King. **1995**. Venom of *Catolaccus grandis* (Hymenoptera : Pteromalidae) and its role in parasitoid development and host regulation. Ann. Entomol. Soc. Am. 88: 800–808.

Moreau, S. J. M., and S. Asgari. **2015**. Venom proteins from parasitoid wasps and their biological functions. Toxins (Basel). 7: 2385–412.

Mrinalini, A. L. Siebert, J. Wright, E. Martinson, D. Wheeler, and J. H. Werren. **2015**. Parasitoid venom induces metabolic cascades in fly hosts. Metabolomics. 11: 350–366.

Parkinson, N., E. H. Richards, C. Conyers, I. Smith, and J. P. Edwards. **2002**. Analysis of venom constituents from the parasitoid wasp *Pimpla hypochondriaca* and cloning of a cDNA encoding a venom protein. Insect Biochem. Mol. Biol. 32: 729–735.

Poirié, M., D. Colinet, and J. L. Gatti. **2014**. Insights into function and evolution of parasitoid wasp venoms. Curr. Opin. Insect Sci. 6: 52–60.

Qian, C., Y. Liu, Q. Fang, Y. Min-Li, S. S. Liu, G. Y. Ye, and Y. M. Li. **2013**. Venom of the ectoparasitoid, *Nasonia vitripennis*, influences gene expression in *Musca domestica* hemocytes. Arch. Insect Biochem. Physiol. 83: 211–231.

Richards, E. H., M. P. Dani, and H. Bradish. **2013**. Immunosuppressive properties of a protein (rVPr1) from the venom of the endoparasitic wasp, *Pimpla hypochondriaca*: mechanism of action and potential use for improving biological control strategies. J. Insect Physiol. 59: 213–222.

Souza, H. D. S., Y. F. Messas, F. Masago, E. F. dos Santos, and J. Vasconcellos-Neto. **2015**. *Paracyphononyx scapulatus* (Hymenoptera, Pompilidae), a koinobiont ectoparasitoid of *Trochosa sp.* (Araneae, Lycosidae). J. Hymenopt. Res. 46: 165–172.

Suzuki, M., and T. Tanaka. **2006**. Virus-like particles in venom of *Meteorus pulchricornis* induce host hemocyte apoptosis. J. Insect Physiol. 52: 602–613.

Takasuka, K., and R. Matsumoto. **2011**. Infanticide by a solitary koinobiont ichneumonid ectoparasitoid of spiders. Naturwissenschaften. 98: 529–536.

Tena, A., A. Kapranas, F. Garcia-Marí, and R. F. Luck. **2008**. Host discrimination, superparasitism and infanticide by a gregarious endoparasitoid. Anim. Behav. 76: 789–799.

Weisel-Eichler, a, G. Haspel, and F. Libersat. **1999**. Venom of a parasitoid wasp induces prolonged grooming in the cockroach. J. Exp. Biol. 202 (Pt 8): 957–64.