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REVIEW

The mechanism utilized by *Toxoneuron nigriceps* in inhibiting the host immune system

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Abstract

Insect parasitic factors of both maternal and embryonic origin allow the endoparasitoid progeny to elude the humoral and cellular immune system responses of their host. Endoparasitoid wasps of lepidopteran larval stages inject, along with venom and ovarian proteins, Polydnavirus particles acting in synergy with all other factors in host regulation for parasitism success.

To date, the molecular mechanisms used by endoparasitoid to circumvent the host immune system are little known. Neverthless, several of these strategies are conserved through the wasp parasitoid species.

Heliothis virescens is a noctuid moth, host of the endophagous parasitoid *Toxoneuron nigriceps*. The first observed effect of parasitism is immune system suppression, as direct consequence of the array of host regulation factors, both of embryonic and maternal origin. This review describes the contribution of all the parasitic components during alterations of the host immune response observed after oviposition by *T. nigriceps*.

Key Words: *Heliothis virescens*, immunomodulation, endoparasitoid, polydnavirus, venom, teratocytes

Introduction

The relationship between parasitoids and their hosts can be very complex, involving physiological interactions at different and multiple levels. This association is the result of a long coevolutionary history that in some cases led to a very intimate interaction and to fine regulation of the host physiology by the parasitoid. In the ectoparasitoids, whose larvae feed on the host body from outside, the parasitism is based on the use of venom, able to paralyse the host, allowing the larvae to benefit. In endoparasitoids, whose larvae feed within the host body, parasitism involves several factors, modulating the physiology of the host, in order to obtain a more suitable environment for the development of juvenile stages of their progeny (Schmidt *et al.*, 2001; Federici and Bigot, 2003; Rivers *et al.,* 2005; Vinson, 2012).

Endoparasitoid insects, belonging to the Hymenoptera order, have developed strategies to parasitise their hosts, through specialised mechanisms generated by long adaptive processes that occurred within host/parasitoid physiological interactions (Vinson and Scott, 1974; Vinson and Iwantsch, 1980; Godfray, 1994; Quicke, 1997). The first evidence for the physiological changes observed in parasitised hosts is the inactivation of humoral and cellular defences, that prevents parasitoid egg melanization and encapsulation (Beck *et al.*, 2000; Asgari *et al.*, 2003a; Zhang *et al.*, 2006). The insect immune system is generally able to respond to the intruders within few minutes after injuries. Parasitoids, as a consequence, have evolved their ability to alter the host defence with different strategies, some of which acting very fast. For this reason, during oviposition, parasitoid females introduce maternal factors such as ovarian fluid into the host body (Asgari and Schmidt, 1994; Webb and Luckhart, 1994, 1996; Tanaka *et al.,* 2002), venom (Beckage and Gelman, 2004; Zhang *et al.,* 2004; Kohler *et al.,* 2007) and proteins that cover the egg surface (Asgari and Schmidt, 1994). All of these factors, along with embryonic ones, necessarily mediate immunosuppression during the early stages of parasitism, contributing to the success of parasitism (Strand and Burke, 2015).

Among maternal factors, polydnaviruses (PDVs) are obligate symbionts of endoparasitoid wasps, attacking exclusively larval stages of their lepidopteran hosts (Webb *et al.,* 2000; Webb and Strand, 2005). PDVs have a double-stranded segmented circular DNA, integrated into the

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genome of the parasitoid. PDVs infect hosts and express their genes in tissues without any replication, inducing alterations in host physiology. These alterations allow parasitoid larvae survival and growth, to finally pupate in silken cocoons (Strand and Burke, 2014). PDVs replicate exclusively in wasp ovaries, where their circular genome is generated from linear DNA copies of wasp chromosomes (Varricchio *et al.*, 1999, Volkoff *et al*., 2010; Dupuy *et al*., 2011) and vertically transmitted to the next wasp generation (Strand, 2010).

In several host/parasitoid systems the maternal and embryonic factors of parasitic origin can suppress the host immune system.

The innate immune system of insects includes several mechanisms that sometimes are arbitrarily divided into humoral and cellular responses, interacting at different levels to provide defence against invading intruders, both pathogens and parasites. The first physical barrier is the tegument, with the cuticle and the epithelium beneath. When an external organism overcomes this barrier and reaches the haemocoel, humoral and cellular defence reactions are specifically and sequentially activated, such as soluble molecules and haemocytes circulating in the haemolymph (Lemaitre and Hoffmann, 2007).

Among the humoral responses, melanization is a very fast reaction due to the activation of serine protease cascade, induced by several types of elicitors (microbial surface molecules). This pathway regulates the coagulation or development of melanine both upon injury and on the surface of the foreign invaders. The production of antimicrobial peptides (Boman *et al.*, 1991; Hoffmann *et al.*, 1993; Hultmark, 1993; Cociancich *et al.*, 1994; Lowenberger, 2001) and reactive intermediates of oxygen or nitrogen (Bogdan *et al.*, 2000; Vass and Nappi, 2001) are also part of the humoral response.

Cellular responses are mediated by different types of haemocytes and include encapsulation against parasitic eggs and larvae, phagocytosis of bacterial and yeast cells and nodulations against grouped cells of pathogens, as bacteria or yeasts (Schmidt *et al.*, 2001; Lavine and Strand, 2002).

A very common effect of parasitism is host immune response suppression. Endoparasitoid wasps belonging to Braconidae and Ichneumonidae families induce significant immunosuppression of parasitised hosts. Host immunosuppression is usually attributed to the wasp's ability to avoid or suppress encapsulation which is the major host defence against parasite egg invasion (Summers and Dib-Hajj, 1995). Eggs and larvae of endoparasitoids are able to evade host immune defences either passively and/or by active suppression of the host immune system (Schmidt *et al.*, 2001; Lavine and Strand, 2002). Host defence regulation by the parasitoid is mediated by female secretions injected during oviposition (venom, PDV, ovarian calyx fluid) and embryonic (teratocyte) factors that may act alone and/or in synergy.

For example, the *Microplitis demolitor* bracovirus (*Md*BV) alone inhibits encapsulation and suppresses other host immune defences, including phagocytosis, melanization of haemolymph and inducible expression of other humoral defence molecules (Strand and Pech, 1995 a, b; Beck and Strand, 2005; Thoetkiattikul *et al.*, 2005; Strand *et al.*, 2006).

For the endoparasitoid *Campoletis sonorensis*, Edson *et al* (1981) showed that purified viable polydnavirus was responsible for suppressing the host's (*Heliothis virescens*) ability to encapsulate the wasp eggs.

A cysteine rich gene from *Campoletis chlorideae* polydnavirus is responsible for the disruption of encapsulation and haemocytes cytoskeleton degradation (Zhang and Wang, 2003).

Early genes of *Cotesia congregata* bracovirus (*Cc*BV) cause haemocyte inactivation and apoptosis (Lavine and Beckage, 1995; Le *et al.,* 2003).

The venom of the braconid *Apanteles glomeratus* (Kitano, 1982) and accessory gland secretions produced by *Pimpla turionella* and the cynipid *Leptopilina heterotoma*, specifically inhibit host encapsulation (Osman, 1978; Rizki and Rizki, 1984; Parkinson *et al.*, 2001). The venom protein Vn50 of *Cotesia rubecula* blocks melanization of its host *Pieris rapae* (Asgari *et al*., 2003b) and a layer of calyx fluid glycoproteins protects the developing wasp during embryogenesis (Asgari and Schmidt, 1994). Also teratocytes (Kitano, 1969, 1974; Vinson, 1972) or the egg itself (Kitano and Nakatsuji, 1978) can produce soluble substances to suppress host immune system.

This review provides an overview of the interaction between the host/parasitoid model system *Heliothis virescens/Toxoneuron nigriceps*, focusing on the immune system inhibition of *H. virescens* by *T. nigriceps* during parasitism.

The host/parasitoid system: *Heliothis virescens/Toxoneuron nigriceps*

Toxoneuron nigriceps (Hymenoptera: Braconidae) is a solitary braconid endoparasitoid wasp of the tobacco budworm larval stages *Heliothis virescens* (Lepidoptera, Noctuidae). During oviposition, *T. nigriceps* females inject into the host larva, along with the egg, the venom and the ovarian calyx fluid that include the ovarian proteins and a symbiotic polydnavirus (PDV) named *T. nigriceps* bracovirus (*Tn*BV) (Fig. 1). Parasitism can occur at all host larval stages and parasitised caterpillars can reach the stage of mature larvae but they fail to pupate (Lewis and Vinson, 1968; Pennacchio *et al*., 1993). As in other endoparasitoid wasps, the integration of parasitic factors of maternal origin (ovarian calyx fluid, PDV and venom) with embryonic origin factors (teratocytes) causes physiological alterations resulting in host developmental arrest, crucial for *T. nigriceps* larvae that grow and finally pupate in silken cocoons (Lewis and Vinson, 1968; Vinson and Iwantsh, 1980).

*Tn*BV

T. nigriceps is associated with a polydnavirus (PDV), *T. nigriceps* bracovirus (*Tn*BV), injected during oviposition together with the egg. The *Tn*BV genome is composed of DNA circles of different

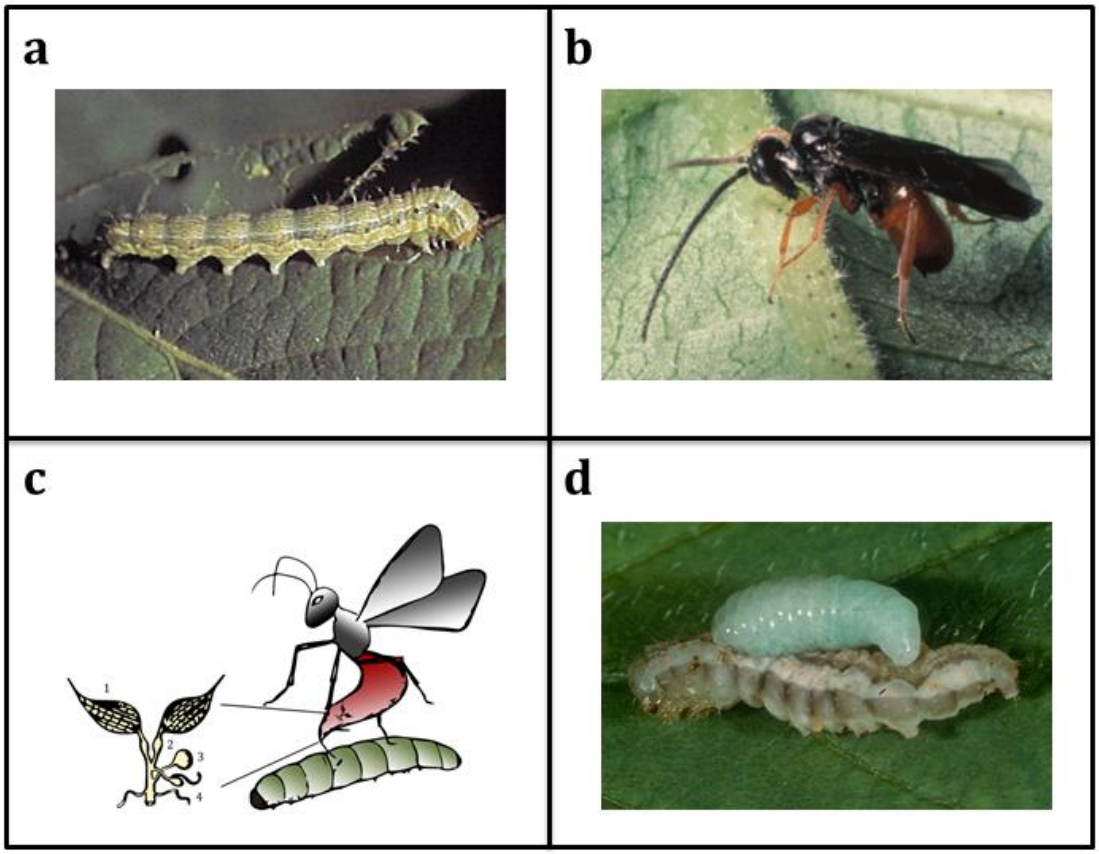

Fig. 1 *Heliothis virescens* (Lepidoptera, Noctuidae) last (fifth) instar larvae, parasitised by *Toxoneuron nigriceps* (Hymenoptera: Braconidae). During parasitisation (c), *T. nigriceps* female (b) injects into *H. virescens* (a - https://commons.wikimedia.org/w/index.php?curid=1215079) the egg, stored in the ovaries (c-1), the ovarian calyx (c-2) fluid, that includes the ovarian proteins and a symbiotic polydnavirus named *T. nigriceps* bracovirus (*Tn*BV), and the venom, stored in the reservoir (c-3) and released by venom glands (c-4). The alteration of the neuroendocrine and the immune systems of the host prevents its final pupation and promotes the parasitoid larva development. This strategy leaves the host alive since the mature parasitoid larva egression (d). Figures b and d were kindly provided by Dr. Vinson lab.

sizes, ranging from 3.4 to 13.3 kb (Fig. 2). The coding sequences include open reading frames (ORFs) encoding putative proteins that for the majority belongs to 4 gene families: ANK (protein with ankyrin repeat motif) (Falabella *et al.*, 2007) PTP (protein tyrosine phosphatase) (Provost *et al.*, 2004; Falabella *et al.*, 2006), UDP (glucose-6-dehydrogenase and sugar transporter) and BEN domain proteins (Falabella *et al.*, unpublished data).

In synergy with other parasitic factors, the symbiotic PDV seems to play the major role in inducing the disruption of host physiology. In particular, the injection and active transcription of *Tn*BV genes are responsible for the alteration of the host neuro-endocrine balance, through the inactivation of host prothoracic glands (PGs) (Tanaka and Vinson, 1991; Pennacchio *et al.*, 1997, 1998 a, b). The consequent block of ecdysteroidogenesis in the last instar larvae inhibits pupation. Although the involvement of both phosphoinositide 3-kinase/protein kinase B/target of rapamycin (PI3K/Akt/TOR) and mitogen-activated protein kinase (MAPK) pathways in prothoracicotropic hormone (PTTH)-stimulated ecdysteroidogenesis in *H. virescens* PGs has been demonstrated (Scieuzo *et al.*, 2018), to date only the effect of *Tn*BV infection on PI3K/Akt/TOR cellular signalling is known (Falabella *et al.*, unpublished data).

The viral genes potentially involved in this process have been identified (Falabella *et al.*, unpublished data). Moreover, through a transcriptomic approach, viral genes differentially expressed in PGs were identified. These genes could be involved in modulating ecdysteroidogenesis pathways.

*Tn*BV gene expression in haemocytes of *H. virescens* parasitised larvae is primarily responsible for inducing immunosuppression.

The exact mechanism of host immune response inactivation by *Tn*BV is not completely elucidated (Varricchio *et al.*, 1999; Pennacchio *et al.*, 2001; Falabella *et al.*, 2003; Malva *et al.*, 2004; Provost *et al.*, 2004; Lapointe *et al.*, 2005). Indeed, the *in vitro* and *in vivo* investigation of the specific function of *Tn*BV genes expressed in *H. virescens* immune cells is difficult. Nevertheless, the functional

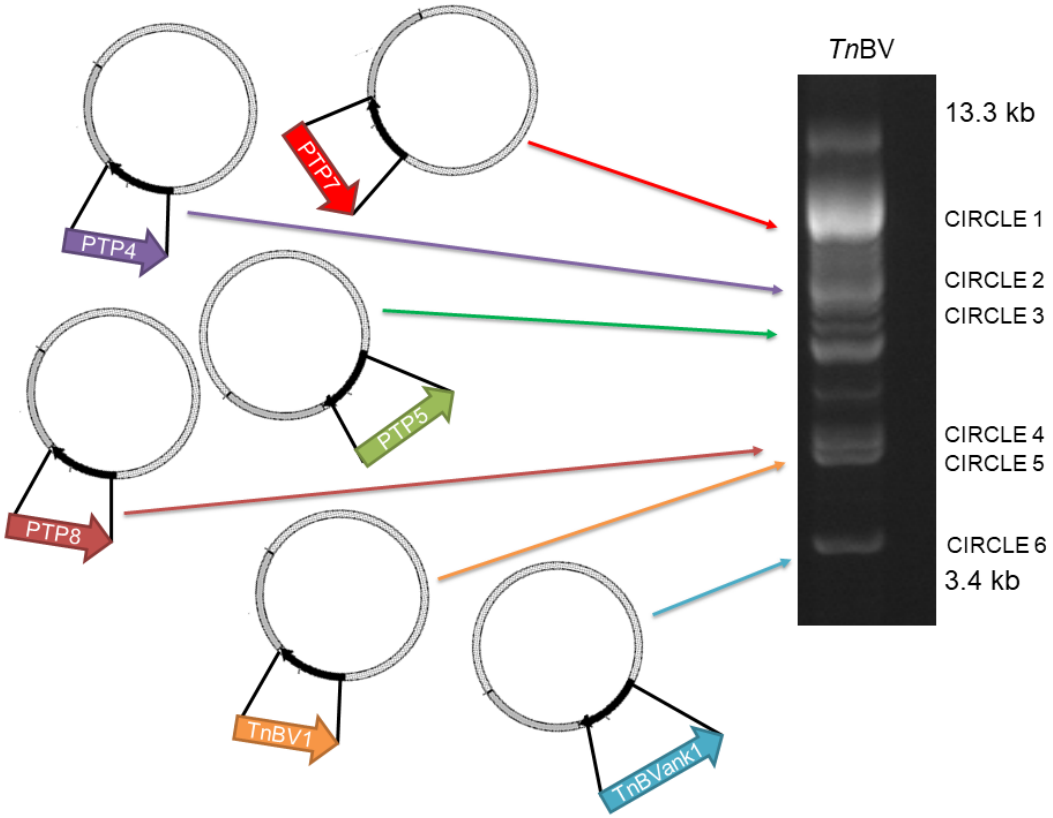

Fig. 2 Viral genes expressed in haemocytes of *Heliothis virescens* parasitised by *Toxoneuron nigriceps.* Gel profile of *Toxoneuron nigriceps* bracovirus (*Tn*BV) showing a segmented genome, with circular double-stranded DNA molecules of different size ranging from 3.4 to 13.3 kilobases. *Tn*BV genes expressed in haemocytes of parasitised *Heliothis virescens* larvae are located on different viral circles (Provost *et al.*, 2004; Falabella *et al.*, unpublished data), conventionally named from 1 to 6, in order from the higher to the lower molecular weight

annotation of several isolated *Tn*BV genes (Fig. 2) may indicate their possible role in disruption of haemocytes-mediated encapsulation reactions by the host (Asgari *et al.*, 1997; Provost *et al.*, 2004).

Among *Tn*BV genes actively expressed in the haemocytes of parasitised larvae, the gene *Tn*BV1 seems to be involved in the host immunosuppression (Lapointe *et al.*, 2005).

In order to characterise the possible involvement of *Tn*BV1 in immunosuppression of *H. virescens* parasitised larvae, this gene was heterologously expressed in various lepidopteran cell lines using different systems and the effects of gene expression on cell morphology and viability were evaluated. *Tn*BV1 induced apoptosis when expressed in Sf21 cells derived from *Spodoptera frugiperda* and when expressed in High Five cells derived from the lepidopteran *Tricoplusia ni* (Lapointe *et al.*, 2005).

However, when *Tn*BV1-recombinant baculovirus was used to *in vivo* infect the host haemocytes, the expression of *Tn*BV1 does not exert any apoptotic effect, leaving the role of *Tn*BV1 expression in parasitised host larvae only partly understood. It is possible that during parasitism *Tn*BV1 protein may interact with other viral or host proteins, inducing the apoptotic pathway in synergy with them. On the other hand, all the effects observed in two different cell lines, both derived from Lepidoptera and expressing *Tn*BV1 alone, overlap with general effects observed in haemocytes of parasitised host. Ferrarese *et al* (2005) demonstrated that following parasitism the total numbers of haemocytes transiently decreased and among them granulocytes showed actin cytoskeleton disruption and loss of adhesion properties. All these evident morphological changes point towards an induction of apoptosis, at least in granulocytes of parasitised host larvae (Lapointe *et al.*, 2005).

The same apoptotic effects were observed in a polyclonal *Drosophila* S2 cell line stably expressing the *Tn*BV viral protein coding by the*Tn*BVank1 gene, and in haemocytes of *H. virescens* larvae, both after natural parasitism and after *Tn*BVank1 *in vivo* transient transfection (Salvia *et al.,* 2017).

*Tn*BVank1 belongs to the *Tn*BV gene family including ankyrin motif proteins (ANKs) (Falabella *et al.*, 2007). The ANK gene family, together with PTP encoding genes, is the largest and most conserved bracovirus gene family (Bitra *et al.*, 2012). These proteins show a high levels of amino acid identity to

the ankyrin motif of the *Drosophila* transcription factor Nuclear -Factor- kappa-light-chain-enhancer of activated B cells (NF-kB) inhibitor (IκB)-related protein cactus (Meng *et al.*, 1999; Falabella *et al.*, 2007). IkB, a member of the Toll and IMD pathways, is part of the immune defence system of insects, and in presence of some key molecules belonging to bacteria and fungi, activates the transcription factor NF-kB, that regulates the expression of several genes involved in immunity, for example the antimicrobial peptides (AMPs) (Dushay *et al.*, 1996; Lemaitre *et al.*, 1996; Han and Ip, 1999; De Gregorio *et al.*, 2001; Uvell and Engstrom, 2003). Three ankyrin-like open reading frames (ORFs) were identified in the *Tn*BV genome and were named *Tn*BVank1, *Tn*BVank2 and *Tn*BVank3 (Falabella *et al.*, 2007). All the *Tn*BVank genes contain the ankyrin motif but the phosphorylable serine residues at the N-terminus, essential for the IKB protein function, are absent (Falabella *et al.*, 2007). Moreover, all of putative proteins encoded by the *Tn*BVank genes lack the C-terminal PEST domain, characteristic in all actives IκB like proteins and essential for protein degradation (Ghosh *et al.*, 1998, Ghosh and Karin, 2002; Webb and Strand, 2005) and for the protein turnover control (Rogers *et al.*, 1986).

On the basis of the predicted structure of *Tn*BVank genes, it was demonstrated that the *Tn*BVank1 protein binds NF-kB/Rel transcription factors of the tumor necrosis factor (TNF)/Toll immune pathway altering the signal transduction cascade (Falabella *et al.*, 2007). Indeed, in parasitised *H. virescens* larvae, after bacterial challenge, the nuclear import of NF-κB was inhibited (Falabella *et al.*, 2007). Moreover, the expression of *Tn*BVank1 gene in human HeLa cells reduced the efficiency of the TNF-α-induced expression of a reporter gene under NF-κB transcriptional control, strongly suggesting that *Tn*BVank1 protein is involved in the suppression of the insect immune response (Bitra *et al.*, 2012; Falabella *et al.*, 2007; Thoetkiattikul *et al.*, 2005). The same function was demonstrated for ANK family members of *Microplitis demolitor* bracovirus (*Md*BV) which, competing with the host IkB for binding NF-kB/Rel, reduce the expression of AMPs (Bitra *et al*, 2012).

The early expression of the *Tn*BVank1 gene (three hours after parasitism) in haemocytes of *H. virescens* last instar larvae and the presence of transcripts several hours after parasitism, corroborate *Tn*BVank1 involvement during early immune response suppression of parasitised host, also through the activation of a kind of cell death attributable to a process of apoptosis (Falabella *et al.*, 2007).

As reported before (Salvia *et al.,* 2017), *Tn*BVANK1 induces cell death in polyclonal *Drosophila* S2 cells, that stably express *Tn*BVank1 and in haemocytes derived from both *H. virescens* parasitised larvae or *in vivo* transfected using *Tn*BVank1. Coimmunoprecipitation and coimmunolocalisation experiments in *Tn*BVank1 polyclonal *Drosophila* S2 cells showed that *Tn*BVANK1 interacts with ALG-2 interacting protein X (Alix). Alix is a multifunctional protein and it was first characterised as interactor of apoptosis-linked gene protein 2 (ALG-2), that is a Ca2+-binding protein necessary for cell death (Missotten *et al.*, 1999).

It was demonstrated that *Tn*BVANK1-Alix interaction is required to induce apoptosis in all cells expressing this gene, both *in vitro* (S2 cells) and *in vivo* (*H. virescens* larval haemocytes). Indeed, upon Alix silencing by RNA interference (RNAi), *Tn*BVANK1 was no longer able to induce apoptosis (Salvia *et al.*, 2017).

Among the gene families present in bracoviruses of different braconid wasp subfamilies, the PTP gene family is probably an ancient component of the ancestral bracovirus of both Microgastrinae and Cardiochilinae subfamilies**.**

PTPs are enzymes that catalyse the phosphorylation of specific protein target at tyrosine residues. Together with protein tyrosine kinase, PTPs regulate the phosphorylation level of cellular proteins (Neel and Tonks, 1997).

The functional characterisation of bracovirus PTPs expressed in their hosts may mean that most of them have a role in the success of parasitism. Although in some cases PTP genes do not codify for functionality active enzymes, they could contribute to host/parasitoid interactions performing different functions, for example binding phosphorylated proteins and inhibiting their activities (Provost *et al.*, 2004).

Bracovirus PTPs are numerous and characterised by a high degree of diversity, regarding also the target substrate, at least for PTPs characterised until now. This heterogeneousness indicates that they may act altering several cell pathways in parasitised host, especially those regulating the host immunity and endocrine balance (Provost *et al.*, 2004).

In particular, regarding parasitised host immune suppression, it is possible to hypothesise that bracovirus PTPs could alter the haemocyte cytoskeleton, strongly reducing their ability to encapsulate the intruders and losing their phagocytic ability. In this last case we could compare the activity of bracovirus PTPs with the ability of some pathogens, bacteria in particular, in using PTPs to alter the control of actin dynamics in infected host cells, resulting in inhibition of phagocytosis (Bleves and Cornelis, 2000; De Vinney *et al.*, 2000; Ernst, 2000; Cornelis, 2002; Gruenheid and Finlay, 2003; Singh *et al.*, 2003; Mustelin *et al.*, 2005). Among bracoviruses, the *Md*BV suppresses *Spodoptera frugiperda* immune system inactivating phagocytosis, encapsulation and melanization reaction of haemolimph (Strand and Pech, 1995a,b; Beck and Strand, 2005; Thoetkiattikul *et al.*, 2005; Strand *et al.*, 2006; Suderman *et al.*, 2008). The putative proteins encoded by *Md*BV include 13 members of PTP gene family, but only 5 PTPs show a potentially active catalytic domain, while the other 8 members of *Md*BV PTPs seem to encode functionally inactive enzymes, since their sequence analysis showed modification not compatible with tyrosine phosphatase activity (Pruijssers and Strand, 2007). Among the potentially active *Md*BV PTPs, PTP-H2 and PTP-H3 are preferentially expressed in host haemocytes and localise to focal adhesions, also

when they are expressed in haemocyte-like cells, such as *Trichoplusia ni* High Five and *Drosophila* S2 cell lines. In these cases, they strongly contribute to disruption of adhesion and inhibition of phagocytosis, complementing the activity of *glc*1.8, another *Md*BV gene with antiadhesive and antiphagocytic properties (Suderman *et al.*, 2008).

Moreover, *Md*BV PTP-H2 also induces apoptosis of Sf21 cells. The transient expression of PTP-H2 in this cell line induced the depolarisation of mitochondrial membrane and caspase-dependent apoptosis (Suderman *et al.*, 2008). On the basis of these results Suderman and colleagues (2008) hypothesised that PTP-H2 contributes to immunosuppression of hosts defences also with this activity.

The *Tn*BV genome includes 13 genes encoding putative classical PTPs (Falabella *et al.*, 2006). All identified PTP genes have a specific domain including 10 conserved motifs found in vertebrates (Andersen *et al.*, 2001). No relation was found with dual-specificity PTPs present in baculovirus or poxvirus (Provost *et al.*, 2004), challenging the hypothesis of a baculovirus as possible progenitor of BVs (Drezen *et al.*, 2003; Federici and Bigot, 2003).

Sequence analysis of the 13 *Tn*BV identified PTPs showed that 8 PTP genes have a complete and potentially active protein tyrosine phosphatase domain. The other 5 PTPs encode incomplete putative proteins or they are interrupted by a stop codon or by a frameshift (Provost *et al.*, 2004).

Among the *Tn*BV gene families, PTP is the largest one. Its members lack introns, similarly to the *Cotesia congregata* bracovirus (*Cc*BV) PTP family (Provost *et al.*, 2004).

Using a multiple approach 4 PTPs were identified as expressed in haemocytes of *H. virescens* parasitised larvae (Falabella *et al.*, 2006; Falabella *et al.*, unpublished data) (Fig. 2).

All PTP cDNAs from *Tn*BV encode putative classical PTPs with cytosolic localisation.

The expression of these PTPs in haemocytes of *H. virescens* parasitised larvae suggests their contribution towards host immune system suppression induced by *Tn*BV. Although no experimental data support this conclusion, we propose *Tn*BV PTPs as responsible for the alteration of cytoskeleton dynamics during the cellular immune response. In particular, this event could occur during encapsulation and phagocytosis, similar to the mechanism adopted by some pathogens that, expressing their PTPs as virulence factors, alter the host cell signal transduction pathway, modifying the actin rearrangements thus inducing inhibition of phagocytosis (Bleves and Cornelis, 2000; De Vinney *et al.*, 2000; Ernst, 2000; Pruijssers and Strand, 2007)

Although similar data were found in case of PTPs belonging to other bracovirus, where PTPs are actually involved in alteration of host immunity, this remains to be verified experimentally.

Venom

The venom of endoparasitod wasps is a mixture of compounds, mainly proteins, produced by venom glands, organs located in the female reproductive system in Hymenoptera (Fig. 1) (Billen, 1987; Dorémus *et al.*, 2013). Venom proteins start to be synthesised during the wasp pupal stages in the venom glands, connected to a reservoir where the venom is collected and stored (Jones and Wozniak, 1991) (Fig. 1). The reservoir is directly attached to the terminal part of the oviduct, where the venom is mixed with the ovarian calyx fluid produced in the swollen base of the ovary (Fig. 1). This fluid is composed of a protein mixture (ovarian proteins) and in case of endoparasitoids of lepidopteran larval stages, of polydnavirus (PDV) particles (Webb, 1998).

Venom is one of the parasitic factor of maternal origin, injected together with the egg and other factors (i.e. ovarian protein and/or PDV) into the host body during parasitism (Moreau and Asgari, 2015).

In synergy with all the parasitic factors, venom acts to assure an adequate environment, allowing the growth of progeny (Beckage and Gelman, 2004; Moreau and Asgari, 2015).

In endoparasitoids associated with PDVs, venom regulates host physiology and no paralytic or lethal effects are observed, in contrast to the effects of venom injected into hosts by ectoparasitoids or by endoparasitoid lacking PDVs (Wharton, 1993; Quistad *et al.*, 1994; Parkinson and Weaver, 1999; Moreau *et al.*, 2002; Nakamatsu and Tanaka, 2003; Webb and Strand, 2005; Asgari, 2006). In many host/parasitoid systems, venom complements the activity of PDVs (Kitano, 1986; Tanaka, 1987) and in several instances some venom components provide protection for the eggs against the host immune system during the period between oviposition and expression of PDV genes (Webb and Luckhart, 1994).

In endoparasitoids, venom can induce developmental alterations, host castration (Digilio *et al.*, 1998, 2000) and almost always plays an immunosuppression role in the host (Webb and Luckhart, 1994; Luckhart and Webb, 1996; Asgari *et al.*, 1998*).*

The venom of *T. nigriceps* plays a fundamental role for the success of parasitism, indeed it was demonstrated that, when venom glands were removed from female wasps, no parasitoids emerged from host larvae, *H. virescens* (Tanaka and Vinson, 1991). On the other hand, *in vitro* experiments on *H. virescens* prothoracic glands (PGs) demonstrated that the *T. nigriceps* bracovirus (*Tn*BV) alone was able to induce the biosynthetic inactivation of PGs (Pennacchio *et al.*, 1998b).

Regarding parasitoid wasp venom composition, it has thus far been analysed in at least 20 species belonging to different families, using different approaches (Vincent *et al.* 2010; Asgari and Rivers, 2011; Doremus *et al.* 2013; Colinet *et al.*, 2013a,b; 2014; Shaina *et al.*, 2016; Yan *et al.*, 2016; Cusumano *et al.*, 2018). The most recent studies combine transcriptomics of venom glands and proteomics of venom to identify the proteins inject by wasps into the hosts during parasitism (Colinet *et al.* 2013b). Among the different proteins identified in parasitoid venoms, many share significant homology with proteins which functions are known.

Simultaneously, no experimental data exist for the vast majority of parasitoid venom proteins, so their specific role in parasitism generally remains unknown. Indeed, most of the available data focus on proteins that are highly abundant or structurally related to genes in databases with predicted functions, but, when venom proteins are studied in a non-model organism, the interpretation of the results is very difficult (Poirié *et al*., 2014). Some of these factors, including serine proteases, metalloproteases or esterases (Asgari and Rivers, 2011) are shared by many wasp species, while others are known from only one or a small number of species. Some enzymes are also present in hosts at very low concentrations, indicating that focusing exclusively on abundant venom components can potentially lead to overlooking factors that could have important roles in parasitism. The mechanism of entry into the host cells and the mode of action of parasitoid venom proteins are unknown, except in a few species whose venoms contain some virus-like particles (VLPs). The best known VLPs belong to *Leptopilina boulardi*. They are produced in the venom gland and injected into host larvae, *Drosophila melanogaster*, contributing to host immunosuppression. Following injection into host larvae, VLPs target haemocytes, leading to apoptosis and/or morphological changes (Poirié *et al*. 2014).

The main venom protein components of *T. nigriceps* have been identified through the combination of next-generation transcriptome sequencing and bottom-up proteomics (Laurino *et al.*, 2016).

This integrated proteomic and transcriptomic approach allows to identify proteins and biological processes also in non-model organisms (Escoubas *et al.*, 2006; Tang *et al.*, 2010; Safavi-Hemami *et al.*, 2014; Labella *et al.*, 2015).

Beside proteins similar to known venom components or annotated proteins (Asgari and Rivers, 2011; Doremus *et al.*, 2013; Moreau and Asgari, 2015), novel proteins, with no similarity in databases, were identified in *T. nigriceps* venom.

Although no functional data are available, we hypothesise on possible involvement of *T. nigriceps* venom components in alteration of host physiology and in particular in host immune system suppression.

A total of 31 different proteins were identified in *T. nigriceps* venom. Some of these proteins resulted similar to already described proteins in venom from other parasitoids, while others resulted identified for the first time in a venom. Among the known proteins, hydrolases, followed by transferases, oxidoreductases, ligases, lyases and isomerases, was the major family similarly to that reported for venom of other parasitoids (Asgari and Rivers, 2011).

It was speculated that some of the identified proteins could be involved in the host immune suppression directly, in synergy to each other and/or to the other parasitic factors (Table 1).

Based on the MEROPS database (http://merops.sanger.ac.uk), the *T. nigriceps* venom metalloproteases belong to the M12 (M12B subfamily) and M13 families (Laurino *et al.*, 2016). These enzymes could be involved in several biological and disease-related processes (Van Goor *et al.*, 2009). Regarding immunosuppression, there seems to be a parallel between the function of different subfamilies of microbial metalloproteases and the venom metalloproteases. Indeed, entomopathogenic microorganisms use metalloproteases in order to suppress the insect cellular defence, inducing the degradation of host protection molecules (Griesch and Vilcinskas, 1998; Liehl *et al.*, 2006).

This observation may indicate that *T. nigriceps* venom metalloproteases might have similar functions in host immune suppression, in addition to their possible activity in host protein and tissue degradation.

Besides, Serine protease homologue (SPH) and Calreticulin were also found in *T. nigriceps* venom (Laurino *et al.*, 2016). These well-studied proteins are distributed in a wide range of organisms including insects, and it has been reported that they inhibit host cell encapsulation and act as immune suppressor factors in insect haemolymph (Choi *et al.*, 2002; Asgari *et al.*, 2003a; Asgari and Schmidt, 2003; Zhang *et al.*, 2004*;* Kanost and Jiang, 2015).

In particular, SPHs have a serine protease catalytic domain but lack one of the amino acid of the catalytic triad, essential for the proteolytic activity (Hedstrom, 2002).

SPHs are frequently present as components of parasitoid venoms (Hedstrom, 2002; De Graaf *et al.*, 2010) where they block the phenoloxidase (PO) cascade at different levels (Zhang *et al.*, 2004), inducing the inhibition of melanization. Possibly the serine protease homologue identified in *T. nigriceps* venom could play a similar crucial role in suppression of host humoral defences.

The identification of a calreticulin-like protein in the venom of *T. nigriceps* is notable. Indeed calreticulins, molecular chaperones acting as lectin and Ca^{2}+-binding proteins, have been previously identified as a component of several venoms of other parasitoids (De Graaf *et al.*, 2010; Zhu *et al.*, 2010; Asgari *et al.*, 2003b). In particular, a calreticulin in the venom of *Cotesia rubecula* blocked *Pieris rapae* haemocyte diffusion, inhibiting the encapsulation response (Zhang *et al.* 2006). The calreticulin present in *T. nigriceps* venom could bind the intracellular calcium modifying its balance and altering all the cellular pathways in which Ca^{2+} is involved, such as apoptosis, inflammation and activation of hydrolytic enzymes.

In addition, a Ci-48 like protein was identified in *T. nigriceps* venom (Laurino *et al.*, 2016), similar to the venom protein Ci-48 previously identified in *Chelonus inanitus* (Vincent *et al.*, 2010) and in *Microplitis demolitor* venom glands (Burke and Strand, 2014). It was speculated that, in all these cases, the protein could have functions in the early phases upon parasitism that generally involve the inhibition of the host's immune response.

Regarding the function of Elongation factor 1-alpha (EF1-alpha) like protein, recovered in *T. nigriceps* venom (Laurino *et al.*, 2016), although its putative function in host/parasitoid interaction is still unknown, a similar protein was found in the venom

Table 1 Proteins identified in the *Toxoneuron nigriceps* protein database venom putatively involved in the alteration of immune system of the host *Heliothis virescens* (from Laurino *et al.,* 2016)

***T. nigriceps* protein involved in immunity suppression**	**Contig**	**Corresponding Acc. N. NCBI protein indicated in annotations obtained by *T. nigriceps* custom-made database**	**Corresponding Acc. N. protein obtained by BlastP search**
Membrane metallo-endopeptidaselike1-like	4489	gij340723203jrefjXP_003399984.1j	E2AFA5
Venom metalloproteinase 3-like	7042	gij665805846jrefjXP_008551135.1j	F4X7T0
Serine protease homolog 90 isoform x1	18408	gij315131321jembjCBM69269.1j	A0A034V0K7
Calreticulin	5341	gij665788653jrefjXP_008559929.1j	Q8IS63
Venom protein ci-48°	18596	gij665819695jrefjXP_008558721.1j	E6ZCK2
Elongation factor 1-alpha	4154	gij665799004jrefjXP_008547400.1j	K7IVS1
Spermine oxidase-like	14112	gij665801233jrefjXP_008548621.1j	V9IIS9
Ovalbumin-related protein x-like	4452	-	Q8IS84

of parasitoid *Leptopilina heterotoma* (Colinet *et al.*, 2013b). Moreover, it was also found as a secreted product in *Leishmania protozoan* parasites, where it seems to be involved in the induction of host macrophage deactivation (Nandan *et al.*, 2002).

Among other *T. nigriceps* venom components possibly involved in host immune suppression, the Spermine oxidase-like (SMO) is a FAD-dependent enzyme able to induce the production of reactive oxygen species. It plays several roles in numerous cell functions, and some of them are compatible with an alteration of immune responses (Cervelli *et al.*, 2012). Although its biological role in venom has not been elucidated yet, it is tempting to speculate a similar mechanism regarding the host immune system.

The Ovalbumin-related protein x-like is a member of Ovalbumin family and belongs to the ovalbumin serine protease inhibitor family (ov-serpin). The serpin are well known proteins in insects because of their role in inhibiting the activation of the PO cascade and thus the melanization production (Kanost and Gorman, 2008). This function could also be performed in *T. nigriceps* venom by this protein (Laurino *et al.*, 2016).

Among the 31 identified proteins of *T. nigriceps* venom, the 8 annotated proteins mentioned above (Table 1) likely play a key role in host immune system suppression. This is in agreement both with the function of several other endoparasitoid venoms and with previous researches reported in case of *T. nigriceps* venom by Tanaka and Vinson (1991).

Even this finding supports possible role of *T. nigriceps* venom in the regulation of host immune system, to confirm what has been speculated so far, functional characterisation of venom components is required.

Teratocytes

Teratocytes are specialised cells deriving from the dissociation of the extraembryonic membrane, named serosa, during parasitoid egg hatching in the host haemolymph (Dahlman, 1990, 1991; Dahlman and Vinson, 1993; de Buron and Beckage, 1997; Ali *et al.*, 2013). Teratocytes are produced only by endoparasitoid wasps in a restricted number of species belonging to Braconidae, Scelionidae, Mymaridae, Trichogrammatidae, Aphelinidae and Platygastridae families (Strand *et al.*, 1986; Dahlman and Vinson, 1993; Quicke, 1997; Hotta *et al.*, 2001; Basio and Kim, 2005).

All known teratocytes, once in the hemolymph, rapidly increase in size without undergoing any cell division and thus becoming highly polyploid. Probably this characteristic optimises their secretory function, in order to provide nutrients to the parasitoid progeny larvae, directly or indirectly (Strand and Wong, 1991). Teratocytes, indeed, release several molecules impacting physiology, development and nutritional suitability of the host. They are involved in several different functions, such as the manipulation of host development, through the inhibition of protein synthesis, or the disruption of the endocrine balance, which often modulates host biochemical changes that are nutritionally relevant for the developing parasitoid larvae. The nutritional role of the teratocytes is more direct and evident in host/parasitoid associations, where these cells perform an extra-oral digestion of selected host tissues, in order to allow the release of nutrients in a suitable form for the developing sister larvae (Falabella *et al.*, 2000; Nakamatsu *et al.*, 2002; Falabella et *al.,* 2005; Grimaldi *et al.*, 2006; Falabella *et al.*, 2009; Caccia *et al.*, 2012; Grossi *et al.*, 2016).

In addition, in several host/parasitoid systems, they contribute to host immunosuppression and/or in host immune system modulation for the success of parasitism (Nakamatsu *et al.*, 2002; Rana *et al.*, 2002; Burke and Strand, 2014; Ali *et al.*, 2015).

In the *Cotesia kariyai* and *Cotesia glomerata*, teratocytes (taken at 4 days post parasitism) seem to play a role in preventing encapsulation during the early stages of parasitism by interfering with the phenoloxidase (PO) cascade. Nevertheless, in *C. kariyai* it is reported that they act in synergy with ovarian calyx fluid and venom: only when all 3 parasitoid factors were injected into unparasitised hosts along with first-instar parasitoid larvae, the encapsulation was inhibited (Kitano *et al.*, 1990; Tanaka and Wago, 1990).

Few works have addressed to the role of *T. nigriceps* teratocytes (Dahlman and Vinson 1993; Pennacchio *et al.*, 1992, 1994a,b; Consoli *et al.*, 2005, 2007; Rossi *et al.*, 2012). Their functions may be nutritive, immunosuppressive, or secretory and may be involved in regulating host development (Dahlman and Vinson, 1993).

It has been reported that they provide an important contribution to prevent *H. virescens* pupation. Indeed, as previously reported in parasitised *H. virescens* last instar larvae, the inactivation of the biosynthetic activity of PGs, induced by *Toxonigriceps nigriceps* Bracovirus (*Tn*BV) infection, results in an evident reduction in ecdysone titre in haemolymph (Tanaka and Vinson, 1991; Pennacchio *et al.*, 1998 a,b). The alteration of host ecdysteroid metabolism is mediated, at least in part, also by *T. nigriceps* teratocytes (Pennacchio *et al.*, 1994b). Indeed, when teratocytes of *T. nigriceps* were injected into unparasitised *H. virescens* 1 day old last instar larvae, they inhibited host pupation (Pennacchio *et al.*, 1992). Results suggested that teratocytes acted by affecting the haemolymph ecdysteroid titre, through inactivation of the 20-hydroxyecdysone, which is converted into polar inactive metabolites (Pennacchio *et al.,* 1994b; Bloch *et al.*, 2002).

To date, only 1 protein has been identified in *T. nigriceps* teratocytes: it is an isolated and functionally characterised putative chitinase (Consoli *et al.*, 2005, 2007; Rossi *et al.*, 2012). Chitinases are well known enzymes that catalyse the hydrolysis of chitin, but they can also be produced as a defence mechanism, as it has been shown by the expression of chitinases in transgenic plants challenged with micro-organisms, nematodes, and insects (Broglie and Broglie, 1993; Lin *et al.*, 1995).

It has been hypothesised that *T. nigriceps* chitinase, released by teratocytes, may either play a putative protective role preventing any possible microbial infection in the host haemocoel, since host-immune defences are suppressed. Moreover, it could promote host cuticle digestion, allowing the parasitoid larva to emerge from the host body, since the parasitoid larva does not possess powerful mandibles at egression (Lewis and Vinson, 1968).

Recently, we established a *de novo* transcriptome of *T. nigriceps* teratocytes (Falabella *et al.*, unpublished data). The functional annotation and analysis of contigs allowed us to identify several transcripts coding for putative proteins possible involved in the success of parasitism, through the modulation of host immunity.

In particular, similarly to the transcriptomic analysis of *Microplitis demolitor* teratocytes, where the expression of several antimicrobial peptides (AMPs) was found (Burke and Strand, 2014), we identified AMPs, belonging to defensins, attacins and lysozyme families, putatively expressed by *T. nigriceps* teratocytes and potentially released in the host haemolymph (Falabella *et al.*, unpublished data). Teratocytes might prevent host infection by pathogen intruders supporting the immunosuppressed host by the production of AMPs.

Moreover, the same analysis allowed us to identify putative proteins belonging to ovalbumin-related X-like, serpin and Rho GAP families (Falabella *et al.*, unpublished data).

Transcriptome analysis of *Cotesia plutellae* teratocytes reported that these cells express several putative immunosuppressive factors, including several serpins and RhoGAPs proteins, involved in the inhibition of the prophenoloxidase (PPO) cascade system, inducing host immunosuppression (Ali and Kim, 2015; Kanost, 1999).

Taken together, this information may indicate that also *T. nigriceps* teratocytes may have a role in host immunosuppression. However, the specific functions remain to be elucidated requiring further studies.

Concluding remarks

Although insects have only an innate immune system, they have evolved several complex strategies to defend themselves against pathogens. On the other hand, hymenopteran parasitoids, especially endoparasitoid, have coevolved an arsenal of factors that allow the physiology of the host to be synergistically regulated, in order to ensure the complete development of their progeny. These parasitic factors induce diverse and complex reactions against the host defence mechanisms. To date, these intriguing processes are still poorly understood. This review reports an overall picture describing the strategy used by *T. nigriceps* in inhibiting the immune system of its host, the lepidopteran *H. virescens* (Fig. 3).

The findings reported in previous works and some preliminary results showed here, highlight the connection and the fine regulation performed by all parasitic factors in host immunity inactivation.

Almost all the identified *T. nigriceps* bracovirus (*Tn*BV) genes are expressed in host haemocytes and, as consequence, can be considered putatively involved in the host immunity alteration (Fig. 2). To date the genome of *Tn*BV is being sequenced and this could lead to the identification of new genes. Once *Tn*BV genome will be functional annotated, we will obtain an overview on the possible roles of putative viral proteins expressed in host tissues will be available. However a functional characterization of all the viral genes will be required. The role of each viral gene will be studied by *in vivo* transfection in *H. virescens* haemocytes as previously reported for *Tn*BVANK1 (Salvia *et al.*, 2017).

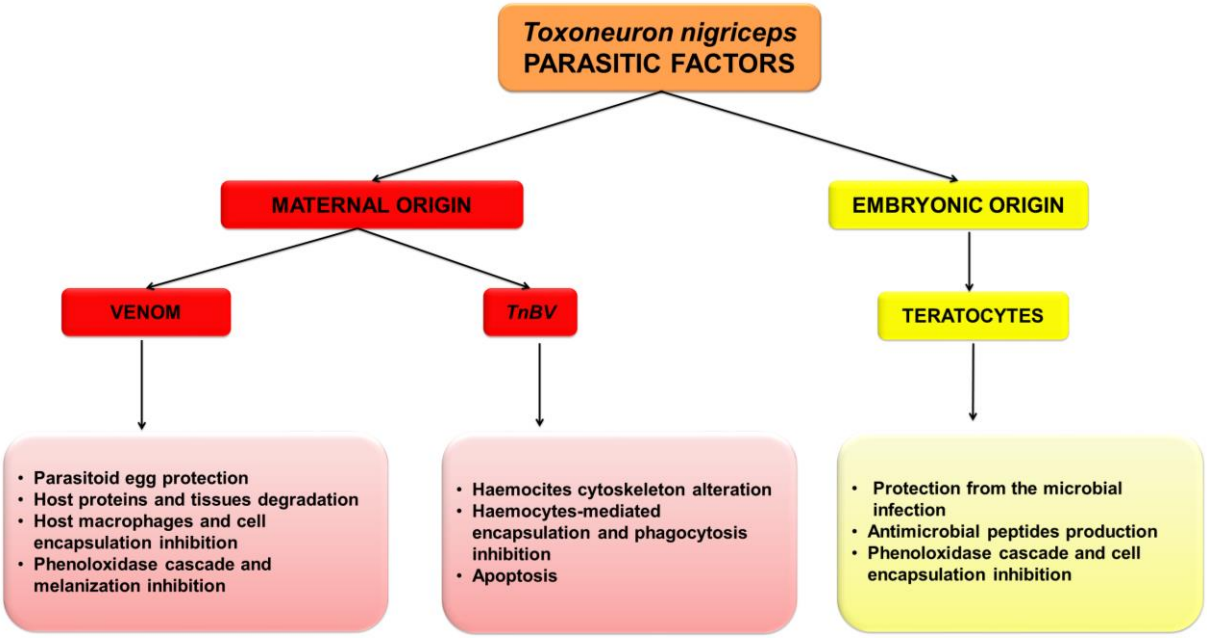

Fig. 3 Overview of *Toxoneuron nigriceps* parasitic factors and their effect on the host immune system. Overall scheme of *T. nigriceps parasitic* factors of maternal and embryonic origin and their role in *Heliothis virescens* immunosuppression.

Moreover, 8 out of 31 venom proteins are putatively responsible for host immune suppression (Table 1). Further analysis of these proteins are required to understand their function and their contribution to the success of parasitism and more specifically to immune suppression. Possible strategies could be the injection of each purified venom protein into the host larvae (Digilio *et al.*, 1998, 2000) and/or with a more advanced approach, the silencing of their expression into *T. nigriceps* venom gland (Lynch and Desplan, 2006; Colinet *et al*., 2014).

At last, also the teratocytes, a parasitic factor of embryonic origin, release molecules clearly involved in the control of the immune response of the parasitised host. The functional annotation of the *de novo* transcriptome may indicate the involvement of antimicrobial peptides released by teratocytes in the regulation of host immunity. Neverthless only the functional characterization of each of them, produced by chemical synthesis or recombinantly, according to their size, will provide most of the information about their role in the host immune system regulation.

What emerges from the information reported in this review is that the regulation of the host immune system by parasitoids is not limited to its inhibition, but also to keep the host alive and in good condition, since it represents the environment and the nutritional resource for the adequate and complete development of the parasitoid offspring.

Concluding, *T. nigriceps* female secretions injected at the oviposition, in synergism with embryonic factors (Fig. 1), induce diverse and partially overlapping mechanisms that inhibit cellular and humoral responses, solving the first problem that parasitoids have to face up: the immune reaction against its egg and the following larval stages (Schmidt *et al*., 2001; Lavine and Strand, 2002).

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References

Ali MR, Lim J, Kim Y. Transcriptome of a specialized extra-embryonic cell, teratocyte, and its host immunosuppressive role revealed by ex vivo RNA interference. Insect. Mol. Biol. 24: 13-28, 2015.

Ali MR, Seo J, Lee D, Kim Y. Teratocyte-secreting proteins of an endoparasitoid wasp, Cotesia plutellae, prevent host metamorphosis by altering endocrine signals. Comp. Biochem. Physiol. A. Mol. Integr. Physiol. 166: 251-62, 2013.

Andersen JN, Mortensen OH, Peters GH, Drake PG, Iversen LF, Olsen OH *et al*. Structural and evolutionary relationships among protein tyrosine phosphatase domains. Mol. Cell. Biol. 21: 7117-36, 2001.

Asgari S. Venom proteins from polydnavirus producing endoparasitoids: their role in host parasite interactions. Arch. Insect Biochem. Physiol. 61: 146-156, 2006.

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

Asgari S, Schmidt O. Passive protection of eggs from the parasitoid, *Cotesia rubecula*, in the host, *Pieris rapae*. J. Insect Physiol. 40: 789-795, 1994.

Asgari S, Schmidt O. Is cell surface calreticulin involved in phagocytosis by insect hemocytes? J. Insect Physiol. 49: 545-550, 2003.

Asgari S, Schmidt O, Theopold U. A polydnavirus-encoded protein of an endoparasitoid wasp is an immune suppressor. J. Gen. Virol. 78: 3061-3070, 1997.

Asgari S, Theopold U, Welby C, Schmidt O. A protein with protective properties against the cellular defense reactions in insects. Proc. Natl. Acad. Sci. USA. 95: 3690-3695, 1998.

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

Asgari S, Zareie R, Zhang G, Schmidt O. Isolation and characterization of a novel venom protein from an endoparasitoid, *Cotesia rubecula* (Hym: Braconidae). Arch. Insect Biochem. Physiol. 53: 92-100, 2003b.

Basio NA, Kim Y. A short review of teratocytes and their characters in *Cotesia plutellae* (Braconidae: Hymenoptera). J. of Asia-Pac. Entomol. 8: 211-217, 2005.

Beck M, Strand MR. Glc1.8 from *Microplitis demolitor* bracovirus induces a loss of adhesion and phagocytosis in insect high five and S2 cells. J Virol. 79: 1861-70, 2005.

Beck M, Theopold U, Schmidt O. Evidence for serine protease inhibitor activity in the ovarian calyx fluid of the endoparasitoid *Venturia cancescens*. J. Insect Physiol. 46: 1275-1283, 2000.

Beckage NE, Gelman DB. Wasp parasitoid disruption of host development: implications for new biologically based strategies for insect control. Annu. Rev. Entomol. 49: 299-330, 2004.

Billen JPJ. New Structural Aspects of the Dufour's and Venom Glands in Social Insects Naturwissenschaften. 74: 340, 1987.

Bitra K, Suderman RJ, Strand MR. Polydnavirus Ank proteins bind NF-kappaB homodimers and inhibit processing of Relish. PLoS Pathog. 8: e1002722, 2012.

Bleves S, Cornelis GR. How to survive in the host: the Yersinia lesson. Microbes Infect. 2: 1451-1460, 2000.

Bloch G, Wheeler D, Robinson G. Endocrine influences on the organization of insect societies. In Pfaff D, Arnold A, Etgen A, Fahrbach S, Moss R, Rubin R, (eds.), Hormones, Brains and Behavior,. Academic Press New York, USA:, Vol. 3 pp 195-237, 2002.

Bogdan C, Rollinghoff M, Diefenbach A. Reactive oxygen and reactive nitrogen intermediates in innate and specific immunity. Curr. Opin. Immunol. 12: 64-76, 2000.

Boman HG, Faye I, Gudmundsson GH, Lee JY, Lidholm DA. Cell-free immunity in Cecropia. A model system for antibacterial proteins. Eur. J. Biochem. 201: 23-31, 1991.

Broglie R, Broglie K. Chitinase gene expression in transgenic plants: a molecular approach to understanding plant defence responses. Phil. Trans. R. Soc. Lond. B. 342: 265-270, 1993.

Burke GR, Strand MR. Systematic analysis of a wasp parasitism arsenal. Mol. Ecol. 23(4): 890-901, 2014.

Caccia S, Grimaldi A, Casartelli M, Falabella P, De Eguileor M, Pennacchio F *et al*. Functional analysis of a fatty acid binding protein produced by *Aphidius ervi* teratocytes. J. Insect Physiol. vol. 58: 621-627, 2012.

Cervelli M, Amendola R, Polticelli F, Mariottini P. Spermine oxidase: ten years after. Amino Acids. 42 : 441-450, 2012.

Choi JY, Whitten MM, Cho MY, Lee KY, Kim MS Ratcliff NA *et al*. Calreticulin enriched as an early-stage encapsulation protein in waxmoth *Galleria mellonella* larvae. Dev. Comp. Immunol. 26: 335-343, 2002.

Cociancich S, Bulet P, Hetru C, Hoffmann JA. The inducible antibacterial peptides of insects. Parasitol. Today 10: 132-139, 1994.

Colinet D, Anselme C, Deleury E, Mancini D, Poulain J, Azéma-Dossat C *et al*. Identification of the main venom protein components of *Aphidius ervi*, a parasitoid wasp of the aphid model *Acyrthosiphon pisum*. BMC Genomics. 15: 342, 2014.

Colinet D, Kremmer L, Lemauf S, Rebuf C, Gatti JL, Poirié M. Development of RNAi in a *Drosophila* endoparasitoid wasp and demonstration of its efficiency in impairing venom protein production. J. Insect Physiol. 63:56-61, 2014.

Colinet D, Mathé-Hubert H, Allemand R, Gatti JL, Poirié M. Variability of venom components in immune suppressive parasitoid wasps: from a phylogenetic to a population approach. J. Insect Physiol. 59: 205-212, 2013a.

Colinet D, Deleury E, Anselme C, Cazes D, Poulain J, Azema-Dossat C *et al*. Extensive inter- and intraspecific venom variation in closely related parasites targeting the same host: the case of *Leptopilina* parasitoids of *Drosophila*. Insect Biochem. Mol. Biol. 43: 601-611, 2013b.

Consoli FL, Brandt SL, Coudron TA, Vinson SB. Host regulation and release of parasitism-specific proteins in the system *Toxoneuron nigriceps-Heliothis virescens.* Comp. Biochem. Physiol. B. Biochem. Mol. Biol. 142(2): 181-191, 2005.

Consoli FL, Lewis D, Keeley L, Vinson SB. Characterization of a cDNA encoding a putative chitinase from teratocytes of the endoparasitoid *Toxoneuron nigriceps*. Entomol. Exp. Appl. 122: 271-278, 2007.

Cornelis GR. Yersinia type III secretion: send in the effectors. J. Cell. Biol. 158: 401-408, 2002.

Cusumano A, Duvic B, Jouan V, Ravallec M, Legeai F, Peri E *et al*. First extensive characterization of the venom gland from an egg parasitoid: structure, transcriptome and functional role. J. Insect Physiol. 22: 68-80, 2018.

Dahlman DL. Evaluation of teratocyte functions: An overview. Arch. Insect Biochem. Physiol. 13: 159-166, 1990.

Dahlman DL. Teratocytes and host/parasitoid interactions. Biol. Control 1: 118-126, 1991.

Dahlman DL, Vinson SB. Teratocytes: developmental and biochemical characteristics. In Beckage NE, Thompson SN, Federici BA (eds) Parasites and pathogens of insects, Academic Press, New York, USA, pp. 145-165, 1993.

De Buron JD, Beckage NE. Developmental changes in teratocytes of the braconid wasp *Cotesia congregate* in larvae of the tobacco hornworm *Manduca sexta*. J. Insect Physiol. 43: 915-930, 1997.

De Graaf DC, Aerts M, Brunain M, Desjardins CA, Jacobs FJ, Werren JH *et al*. Insight into the venom composition of the ectoparasitoid wasp *Nasonia vitripennis* from bioinformatics and proteomic studies. Insect Mol. Biol. 19, 11-26, 2010.

De Gregorio E, Spellman PT, Rubin GM, Lemaitre B. Genome-wide analysis of the *Drosophila* immune response by using oligonucleotide microarrays. Proc. Natl. Acad. Sci. USA 98: 12590-12595, 2001.

De Vinney I, Steele-Mortimer I, Finlay BB. Phosphatases and kinases delivered to the host cell by bacterial pathogens. Trends Microbiol. 8: 29-33, 2000.

Digilio MC, Isidoro N, Tremblay E, Pennacchio F. Host castration by *Aphidius ervi* venom proteins. J. Insects Physiol. 46, 1041-1050, 2000.

Digilio MC, Pennacchio F, Tremblay E. Host regulation effects of ovary fluid and venom of *Aphidius ervi* (Hymenoptera: Braconidae). J. Insect Physiol. 44: 779-84, 1998.

Dorémus T, Urbach S, Jouan V, Cousserans F, Ravallec M, Demettre E *et al*. Venom gland extract is not required for successful parasitism in the polydnavirus-associated endoparasitoid *Hyposoter didymator* (Hym. Ichneumonidae) despite the presence of numerous novel and conserved venom proteins. Insect Biochem. Mol. Biol. 43: 292-307, 2013.

Drezen JM, Provost B, Espagne E, Cattolico L, Dupuy C, Poirié M *et al*. Polydnavirus genome: integrated vs. free virus. J. Insect Physiol. 49: 407-417, 2003.

Dupuy C, Periquet G, Serbielle C, Bézier A, Louis F, Drezen JM. Transfer of a chromosomal Maverick to endogenous bracovirus in a parasitoid wasp. Genetica. 139: 489-496, 2011.

Dushay MS, Asling B, Hultmark D. Origins of immunity: Relish, a compound Rel-like gene in the antibacterial defense of *Drosophila*. Proc. Natl. Acad. Sci. USA 93: 10343-10347, 1996.

Edson KM, Vinson SB, Stoltz DB, Summers MD. Virus in a parasitoid wasp: suppression of the cellular immune response in the parasitoid's host. Science. 211: 582-583, 1981.

Ernst JD. Bacterial inhibition of phagocytosis. Cell Microbiol. 2:379–386, 2000.

Escoubas P, Sollod B, King GF. Venom landscapes: mining the complexity of the spider venoms via a combined cDNA and mass spectrometric approach. Toxicon. 47: 650, 2006.

Falabella P, Caccialupi P, Varricchio P, Malva C, Pennacchio F. Protein Tyrosine Phosphatases of *Toxoneuron nigriceps* bracovirus as potential disrupters of host prothoracic glands. Arch. Insect Biochem. Physiol. 61: 157-169, 2006.

Falabella P, Perugino G, Caccialupi P, Riviello L, Varricchio P, Tranfaglia A *et al*. A novel fatty acid binding protein produced by teratocytes of the aphid parasitoid *Aphidius ervi*. Insect Mol. Biol. 14: 195-205, 2005.

Falabella P, Riviello L, De Stradis ML, Stigliano C, Varricchio P, Grimaldi A *et al*. *Aphidius ervi* teratocytes release an extracellular enolase. Insect Biochem. Mo.l Biol, 39: 801-813, 2009.

Falabella P, Varricchio P, Gigliotti S, Tranfaglia A, Pennacchio F, Malva C. *Toxoneuron nigriceps* polydnavirus encodes a putative aspartyl protease highly expressed in parasitized host larvae. Insect Mol. Biol. 12: 9-17. 2003.

Falabella P, Varricchio P, Provost B, Espagne E, Ferrarese R, Grimaldi A *et al*. Characterization of the IκB-like gene family in polydnaviruses associated with wasps belonging to different Braconid subfamilies. J. Gen. Virol. 88: 92-104, 2007.

Falabella P, Tremblay E, Pennacchio F. Host regulation by the aphid parasitoid *Aphidius ervi*: the role of teratocytes. Ent. Exp. Appl. 97: 1-9, 2000.

Federici BA, Bigot Y. Origin and evolution of polydnaviruses by symbiogenesis of insect DNA viruses in endoparasitic wasps. J. Insect Physiol. 49: 419-432, 2003.

Ferrarese R, Brivio M, Congiu T, Falabella P, Grimaldi A, Mastore M *et al*. Early suppression of immune response in *Heliothis virescens* larvae by the endophagous parasitoid *Toxoneuron nigriceps*. Inver. Surv. J. 2: 60-68, 2005.

Ghosh S, Karin M. Missing pieces in the NF-kappaB puzzle. Cell 109: 81-96, 2002.

Ghosh S, May MJ, Kopp EB. NF-kappa B and Rel proteins: evolutionarily conserved mediators of immune responses. Annu. Rev. Immunol. 16: 225-260, 1998.

Godfray HCJ. Parasitoids: Behavioral and Evolutionary Ecology. Princeton University Press Books, Princeton. 1994.

Griesch J, Vilcinskas A. Proteases released by entomopathogenic fungi impair phagocytic activity, attachment and spreading of plasmatocytes isolated from hemolymph of the greater wax moth *Galleria mellonella*. Biocontr. Sci. Technol. 8: 517-531, 1998.

Grimaldi A, Caccia S, Congiu T, Ferrarese R, Tettamanti G, Rivas-Pena M *et al.* Structure and function of the extraembryonic membrane persisting around the larvae of the parasitoid

Toxoneuron nigriceps. J. Insect Physiol. 52: 870-880, 2006.

Grossi G, Grimaldi A, Cardone RA, Monne´ M, Reshkin SJ, Girardello R *et al.* Extracellular matrix degradation via enolase/plasminogen interaction: Evidence for a mechanism conserved in Metazoa. Biol. Cell. 108: 1-18, 2016.

Gruenheid S, Finlay BB. Microbial pathogenesis and cytoskeletal function. Nature. 422: 775-781, 2003.

Han ZS, Ip YT. Interaction and specificity of Rel-related proteins in regulating *Drosophila* immunity gene expression. J. Biol. Chem. 274: 21355-21361, 1999.

Hedstrom L. Serine protease mechanism and specificity. Chem. Rev. 102: 4501-4524, 2002.

Hoffmann JA, Hetru C, Reichhart JM. The humoral antibacterial response in *Drosophila*. FEBS Lett. 325: 63-66, 1993.

Hotta M, Okuda T, Tanaka T. *Cotesia kariyai* teratocytes: growth and development. J. Insect Physiol. 47: 31-41, 2001.

Hultmark D. Immune reactions in *Drosophila* and other insects: a model for innate immunity. Trends Genet. 9: 178-183, 1993.

Jones D, Wozniak M. Regulatory mediators in the venom of Chelonus sp.: their biosynthesis and subsequent processing in homologous and heterologous systems. Biochem. Biophys. Res. Commun. 178, 213-220, 1991.

Kanost MR. Serine proteinase inhibitors in arthropod immunity. Dev Comp Immunol. 23: 291-301, 1999.

Kanost MR, Gorman MJ. Phenoloxidases in insect immunity. In: Beckage, NE (ed), Insect immunology; Academic Press, San Diego, USA, USA. pp. 69-96, 2008.

Kanost MR, Jiang H. Clip-domain serine proteases as immune factors in insect hemolymph. Curr. Opin. Insect Sci. 11, 47-55, 2015.

Kitano H. Experimental studies on the parasitism of *Apanteles glomeratus* L. with special reference to its encapsulation inhibiting capacity. Bull. Tokyo Gakuge Univ. 21: 95-136, 1969.

Kitano H. Effects of the parasitization of a braconid, *Apanteles*, on the blood of its host, *Pieris*. J. Insect Physiol. 20: 315-327, 1974.

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

Kitano H. The role of *Apanteles glomeratus* venom in the defensive response of its host, *Pieris rapae* crucivora. J. Insect Physiol. 32, 369-375, 1986.

Kitano H., Nakatsuji N. Resistance of *Apanteles* eggs to the haemocytic encapsulation by their habitual host. J. Insect Physiol. 24: 261-271, 1978.

Kitano H, Wago H, Arakawa T. Possible role of teratocytes of the gregarious parasitoid, *Cotesia (=Apanteles) glomerata* in the suppression of phenoloxidase activity in the larval host, *Pieris rapae* crucivora. Arch. Insect Biochem. Physiol. 13: 177-185, 1990.

Kohler LJ, Carton Y, Mastore M, Nappi AJ. Parasite suppression of the oxidations of eumelanin precursors in *Drosophila melanogaster*. Arch. Insect Biochem. Physiol. 66: 64-75, 2007.

Labella C, Kanawati B, Vogel H, Schmitt-Kopplin P, Laurino, Bianco G *et al.* . Identification of two Arginine Kinase forms of endparasitoid *Leptomastix dactylopii* venom by bottom up-sequence tag approach. J. Mass Spectrom. 50: 756-765, 2015.

Lapointe R, Wilson R, Vilaplana L, O'reilly DR, Falabella P, Douris V *et al*. Expression of a *Toxoneuron nigriceps* polydnavirus-encoded protein causes apoptosis-like programmed cell death in lepidopteran insect cells. J. Gen. Virol. 86: 963-971, 2005.

Laurino S, Grossi G, Pucci P, Flagiello A, Bufo SA, Bianco G *et al*. Identification of major *Toxoneuron nigriceps* venom proteins using an integrated transcriptomic/proteomic approach. Insect Biochem. Mol. Biol. 76: 49-61, 2016.

Lavine MD, Beckage NE. Polydnaviruses: potent mediators of host insect immune dysfunction. Parasitol. Today. 11, 368-378, 1995.

Lavine MD, Strand MR. Insect hemocytes and their role in immunity. Insect Biochem. Mol. Biol. 32: 1295-1309, 2002.

Le NT, Asgari S, Amaya K, Tan FF, Beckage NE. Persistence and expression of *Cotesia congregata* polydnavirus in host larvae of the tobacco hornworm, *Manduca sexta*. J. Insect Physiol. 49: 533-543, 2003.

Lemaitre B, Hoffmann J. The host defense of *Drosophila melanogaster*. Annu. Rev. Immunol. 25: 697-743, 2007.

Lemaitre B, Nicolas E, Michaut L, Reichhart JM, Hoffmann JA. The dorsoventral regulatory gene cassette spatzle/Toll/cactus controls the potent antifungal response in *Drosophila* adults. Cell. 86: 973-983, 1996.

Lewis WJ, Vinson SB. Egg and larval development of *Cardiochiles nigriceps*. Ann. Entomol. Soc. Am. 61: 561-565, 1968.

Liehl P, Blight M, Vodovar N, Boccard F, Lemaitre B. Prevalence of local immune response against oral infection in a *Drosophila/Pseudomonas* infection model. PLoS Pathog. 2: e56, 2006.

Lin W, Anuratha CS, Datta K, Potrykus I, Muthukrishnan S, Datta SK. Genetic engineering of rice for resistance to sheath blight. Bio/Technol. 13: 686-691, 1995.

Lynch JA, Desplan C. A method for parental RNA interference in the wasp *Nasonia vitripennis*. Nat Protoc. 1:486-94, 2006.

Lowenberger CA. Form, function and phylogenetic relationships of mosquito immune peptides. Adv. Exp. Med. Biol. 484: 113-129, 2001.

Luckhart S, Webb BA. Interaction of a wasp ovarian protein and polydnavirus in host immune suppression. Dev. Comp. Immunol. 20: 1-21, 1996.

Malva C, Varricchio P, Falabella P, La Scaleia R, Graziani F, Pennacchio F. Physiological and molecular interaction in the host-parasitoid system *Heliothis virescens-Toxoneuron nigriceps*: current status and future

perspectives. Insect Biochem. Mol. Biol. 34: 177-183, 2004.
Meng X, Khanuja BS, Ip YT. Toll receptor-mediated *Drosophila* immune response requires Dif, an NF-B factor. Genes Dev. 13, 792-797, 1999.
Missotten M, Nichols A, Rieger K, Sadoul R. Alix, a novel mouse protein undergoing calcium-dependent interaction with the apoptosis-linked-gene 2 (ALG-2) protein. Cell Death Differ. 6: 124-129, 1999.
Moreau SJ, Asgari S. Venom proteins from parasitoid wasps and their biological functions. Toxin Rev. 7: 2385-2412, 2015.
Moreau SJ, Dingremont A, Doury G, Giordanengo P. Effects of parasitism by *Asobara tabida* (Hymenoptera: Braconidae) on the development, survival and activity of *Drosophila melanogaster* larvae. J. Insect Physiol. 48: 337-347, 2002.
Mustelin T, Vang T, Bottini N. Protein tyrosine phosphatases and the immune response. Nat. Rev. Immunol. 5: 43-57, 2005.
Nakamatsu Y, Fujii S, Tanaka T. Larvae of an endoparasitoid *Cotesia kariyai* (Hymenoptera: Braconidae), feed on the host fat body directly in the second stadium with the help of teratocytes. J. Insect Physiol. 48: 1041-1052, 2002.
Nakamatsu Y, Tanaka T. Venom of ectoparasitoid, *Euplectrus* sp. near plathypenae (Hymenoptera: Eulophidae) regulates the physiological state of *Pseudaletia separata* (Lepidoptera: Noctuidae) host as a food resource. J. Insect Physiol. 49: 149-159, 2003.
Nandan D, Yi T, Lopez M, Lai C, Reiner NE. Leishmania EF-1α activates the Src homology 2 domain containing tyrosine phosphatase SHP-1 leading to macrophage deactivation. J. Biol. Chem. 277: 50190-50197, 2002.
Neel BG, Tonks NK. Protein tyrosine phosphatases in signal transduction. Curr. Opin. Cell Biol. 9: 193-204, 1997.
Osman SE. Die wirkung der sekrete der weiblichen genitalanhangsdrusen von *Pimpla turionellae* L. (Hym., ichneumonidae) auf die hamocyten und die einkapselungsreaktion von wirtspuppen. Z. Parasitenkd. 57: 89-100, 1978.
Parkinson N, Smith I, Weaver R, Edwards JP. A new form of arthropod phenoloxidase is abundant in venom of the parasitoid wasp *Pimpla hypochondriaca*. Insect Biochem. Mol. Biol. 31, 57-63, 2001.
Parkinson N, Weaver RJ. Noxious components of venom from the pupa-specific parasitoid *Pimpla hypochondriaca*. J. Invertebr. Pathol. 73: 74, 1999.
Pennacchio F, Vinson SB, Tremblay E. Host regulation effects on *Heliothis virescens* (F.) larvae induced by teratocytes of *Cardiochiles nigriceps* Viereck (Lepidoptera, Noctuidae-Hymenoptera, Braconidae). Arch. Insect Biochem. Physiol. 19: 177-192, 1992.
Pennacchio F, Vinson SB, Tremblay E. Growth and development of *Cardiochiles nigriceps* (V.) (Hymenoptera, Braconidae) larvae and their synchronization with some changes of the hemolymph composition of their host, *Heliothis virescens* (F.) (Lepidoptera, Noctuidae). Arch. Insect Biochem. Physiol. 24: 65-77, 1993.
Pennacchio F, Vinson SB, Tremblay E. Morphology and ultrastructure of the serosal cells (teratocytes) in *Cardiochiles nigriceps* Viereck (Hymenoptera: Braconidae) embryos. Int. J. Insect Morphol. Embryol. 23: 93-104, 1994a.
Pennacchio F, Vinson SB, Tremblay E, Ostuni A. Alteration of ecdysone metabolism in *Heliothis virescens* (F.) (Lepidoptera, Noctuidae) larvae induced by *Cardiochiles nigriceps* Viereck (Hymenoptera Braconidae) teratocytes. Insect Biochem. Mol. Biol. 24: 383-394, 1994b.
Pennacchio F, Falabella P, Vinson SB. Regulation of *Heliothis virescens* prothoracic glands by *Cardiochiles nigriceps* polydnavirus. Arch. Insect Biochem. Physiol. 38: 1-10, 1998a.
Pennacchio F, Malva C, Varricchio P, Sordetti R, Falabella P, Vinson BS. Prothoracic gland inactivation in *Heliothis virescens* (F.) (Lepidoptera: Noctuidae) larvae parasitized by *Cardiochiles nigriceps* Viereck (Hymenoptera: Braconidae). J. Insect Physiol. 44: 845-857, 1998b.
Pennacchio F, Malva C, Vinson SB. Regulation of host endocrine system by the endophagous braconid *Cardiochiles nigriceps* and its polydnavirus. In: Edwards JP, Weaver RJ. (eds), Endocrine Interactions of Insect Parasites and Pathogens. BIOS, Oxford, UK. pp. 123-132, 2001.
Pennacchio F, Sordetti R, Falabella P, Vinson SB. Biochemical and ultrastructural alterations in prothoracic glands of *Heliothis virescens* (F.) (Lepidoptera: Noctuidae) last instar larvae parasitized by *Cardiochiles nigriceps* Viereck (Hymenoptera: Braconidae). Insect Biochem. Mol. Biol. 27: 439-450, 1997.
Poirié M, Colinet D, Gatti JL. Insights into function and evolution of parasitoid wasp venoms. Curr. Opin. Insect Sci. 6: 52-60, 2014.
Provost B, Varricchio P, Arana E, Espagne E, Falabella P, Huguet E *et al*. Bracoviruses contain a large multigenic family coding for Protein Tyrosine Phosphatases. J.Virol. 78: 13090-13103, 2004.
Pruijssers AJ, Strand MR. PTP-H2 and PTP-H3 from *Microplitis demolitor* bracovirus localize to focal adhesions and are antiphagocytic in insect immune cells. J Virol. 81: 1209-1219, 2007.
Quicke DLJ. Parasitic Wasp. Chapman and Hall. Cambridge. 1997.
Quistad GB, Nguyen Q, Bernasconi P, Leisy DJ. Purification and characterization of insecticidal toxins from venom glands of the parasitic wasp, *Bracon hebetor*. Insect Biochem. Mol. Biol. 24: 955-961, 1994.
Rana RL, Dahlman DL, Webb BA. Expression and characterization of a novel teratocyte protein of the braconid, *Microplitis croceipes* (cresson). Insect Biochem Mol Biol. 32: 1507-1516, 2002.
Rivers DB, Crawley T, Bauser H. Localization of intracellular calcium release in cells injured by venom from the ectoparasitoid *Nasonia vitripennis* (Walker) (Hymenoptera:

Pteromalidae) and dependence of calcium mobilization on G-protein activation. J. Insect Physiol. 51: 149-160, 2005.

Rizki RM, Rizki TM. Selective destruction of a host blood cell type by a parasitoid wasp. Proc. Natl. Acad. Sci. USA. 81: 6154-6158, 1984.

Rogers S, Wells R, Rechsteiner M. Amino acid sequences common to rapidly degraded proteins: the PEST hypothesis. Science. 234: 364-368, 1986.

Rossi GD, Labate MTV, Labate CA, Vinson SB, Consoli FL. Characterization of a *Toxoneuron nigriceps* (Viereck) (Hymenoptera: Braconidae) – derived chitinase and its potential for pest control. Pestic. Biochem. Phys. 104: 96-102, 2012.

Safavi-Hemami H, Hu H, Gorasia DG, Bandyopadhyay PK, Veith PD, Young ND *et al*. Combined proteomic and transcriptomic interrogation of the venom gland of *Conus geographus* uncovers novel components and functional compartmentalization. Mol. Cell. Proteomics 13: 938-953, 2014.

Salvia R, Grossi G, Amoresano A, Scieuzo C, Nardiello M, Giangrande C *et al*. The multifunctional polydnavirus *Tn*BVANK1 protein: impact on host apoptotic pathway. Sci Rep. 7: 11775, 2017.

Schmidt O, Theopold M, Strand MR. Innate immunity and its evasion and suppression by hymenopteran endoparasitoids. Bioessays 23: 344-351, 2001.

Scieuzo C, Nardiello M, Salvia R, Pezzi M, Chicca M, Leis M *et al*. Ecdysteroidogenesis and development in Heliothis virescens (Lepidoptera: Noctuidae): Focus on PTTH-stimulated pathways. J. Insect Physiol. 15: 57-67, 2018.

Shaina H, UlAbdin Z, Webb BA, Arif MJ, Jamil A. De novo sequencing and transcriptome analysis of venom glands of endoparasitoid *Aenasius arizonensis* (Girault) (=*Aenasius bambawalei* Hayat) (Hymenoptera, Encyrtidae). Toxicon. 121: 134-144, 2016.

Singh R, Rao V, Shakila H, Gupta R, Khera A, Dhar N *et al*. Disruption of mptpB impairs the ability of *Mycobacterium tuberculosis* to survive in guinea pigs. Mol. Microbiol. 50: 751-762, 2003.

Strand MR. Polydnaviruses. In: Asgari S, Johnson, KN (eds) Insect Virology. Caister Academic Press, Norwich, UK. pp.171-197. 2010.

Strand MR, Beck MH, Lavine MD, Clark KD. *Microplitis demolitor* bracovirus inhibits phagocytosis by hemocytes from *Pseudoplusia includens*. Arch. Insect Biochem. Physiol. 61: 134-145, 2006.

Strand MR, Burke GR. Polydnaviruses: nature's genetic engineers. Annu. Rev. Virol. 1: 333-354, 2014.

Strand MR, Burke GR. Polydnaviruses: From discovery to current insights. Virology. 479-480: 393-402, 2015.

Strand MR, Meola SM, Vinson SB. Correlating pathological symptoms of *Heliothis virescens* eggs with development of the parasitoid *Telenomus heliothidis*. J. Insect Physiol. 32: 389-402, 1986.

Strand MR, Pech LL. Immunological basis for compatibility in parasitoid-host relationships. Annu. Rev .Entomol. 40: 31-56, 1995a.

Strand MR, Pech LL. *Microplitis demolitor* polydnavirus induces apoptosis of a specific haemocyte morphotype in *Pseudoplusia includens*. J. Gen. Virol. 76: 283-291, 1995b.

Strand MR, Wong EA. The growth and role of *Microplitis demolitor* teratocytes in parasitism of *Pseudoplusia includens*. J. Insect Physiol. 37: 503-515, 1991.

Suderman RJ, Pruijssers AJ, Strand MR. Protein tyrosine phosphatase-H2 from a polydnavirus induces apoptosis of insect cells. J Gen Virol. 89: 1411-1420, 2008.

Summers MD, Dib-Hajj SD. Polydnavirus-facilitated endoparasite protection against host immune defenses. Proc Natl Acad Sci USA. 92: 29-36, 1995.

Tanaka K, Matsumoto H, Hayakawa Y. Detailed characterization of polydnavirus immunoevasive proteins in an endoparasitoid wasp. Eur. J. Biochem. 269: 2557-2566, 2002.

Tanaka T. Calyx and venom fluids of *Apanteles kariyai* (Hymenoptera: Braconidae) as factors that prolong larval period of the host, *Pseudaletia separata* (Lepidoptera: Noctuidae). Ann. Entomol. Soc. Am. 80: 530-533, 1987.

Tanaka T, Vinson SB. Depression of prothoracic gland activity of *Heliothis virescens* by venom and calyx fluids from the parasitoid, Cardiochiles nigriceps. J. Insect Physiol. 37: 139-144, 1991.

Tanaka T, Wago H. Ultrastructural and functional maturation of teratocytes of *Apanteles kariyai*. Arch. Insect Biochem. Physiol. 13: 187-197, 1990.

Tang X, Zhang Y, Hu W, Xu D, Tao H, Yang X *et al*. Molecular diversification of peptide toxins from the *Tarantula Haplopelma* hainanum (Ornithoctonus hainana) venom Based on transcriptomic, peptidomic, and genomic analyses. J. Proteome Res. 9: 2550, 2010.

Thoetkiattikul H, Beck MH, Strand MR. Inhibitor kb-like proteins from a polydnavirus inhibit NF-kB activation and suppress the insect immune response. Proc. Natl. Acad. Sci. USA. 102: 11426-11431, 2005.

Uvell H, Engstrom Y. Functional characterization of a novel promoter element required for an innate immune response in *Drosophila*. Mol. Cell. Biol. 23: 8272-8281, 2003.

Van Goor H, Melenhorst WB, Turner AJ, Holgate ST. Adamalysins in biology and disease. J. Pathol. 219: 277-286, 2009.

Varricchio P, Falabella P, Sordetti R, Graziani F, Malva C, Pennacchio F. *Cardiochiles nigriceps* polydnavirus: molecular characterization and gene expression in parasitized *Heliothis virescens* larvae. Insect Biochem. Mol. Biol. 29(12): 1087-1096, 1999.

Vass E, Nappi AJ. Developmental and immunological aspects of *Drosophila*-parasitoid relationships. J. Parasitol. 86: 1259-1270, 2001.

Vincent B, Kaeslin M, Roth T, Heller M, Poulain J, Cousserans F *et al*. The venom composition of

the parasitic wasp *Chelonus inanitus* resolved by combined expressed sequence tags analysis and proteomic approach. BMC Genomics. 11: 693, 2010.

Vinson SB. Factors involved in successful attack on *Heliothis virescens* by the parasitoid *Cardiochiles nigriceps*. J. Invert. Path. 20: 118-123, 1972.

Vinson SB, Iwantsch GF. Host suitability for insect parasitoids. Annu. Rev. Entomol. 25: 397-419, 1980.

Vinson SB. Foreword - The Discovery of Polydnaviruses and the Influence of Dr. George Salt in: Beckage NE, Drezen JM (eds), Parasitoid Viruses: Symbionts and Pathogens, Academic Press, Cambridge, USA, pp: xi-xv., 2012.

Vinson SB, Scott JR. Parasitoid egg shell changes in a suitable and unsuitable host. J. Ultrastruct. Res. 47: 1-15, 1974.

Volkoff AN, Jouan V, Urbach S, Samain S, Bergoin M, Wincker P, Demettre E, *et al.* Analysis of Virion Structural Components Reveals Vestiges of the Ancestral Ichnovirus Genome. PLoS Pathog. 6: e1000923, 2010.

Webb BA. Polydnavirus biology, genome structure, and evolution. In: Miller LK, Ball LA. (eds), The Insect Viruses. Plenum Publishing Corporation, New York USA. pp. 105-139, 1998.

Webb BA, Beckage NE, Hayakawa Y, Krell PJ, Lanzrein B, Strand MR et al. Polydnaviridae, in: Regenmortel MHV *et al*. (eds.) Virus Taxonomy. Academic Press, San Diego. pp. 253-259, 2000.

Webb BA, Luckhart S. Evidence for an early immunosuppressive role for related *Campoletis sonorensis* venom and ovarian proteins in Heliothis virescens. Arch. Insect Biochem. Physiol. 26: 147-163, 1994.

Webb BA, Luckhart S. Factors mediating short- and long-term immune suppression in a parasitized insect. J. Insect Physiol. 42: 33-40, 1996.

Webb BA, Strand MR. The biology and genomics of polydnaviruses. in Gilbert LI, Iatrou K, Gill SS (eds) Comprehensive Molecular Insect Science, Pergamon,Oxford, UK. pp: 260-323, 2005.

Wharton RA. Bionomics of the braconidae. Annu. Rev. Entomol. 38: 121-143, 1993.

Yan Z, Fang Q, Wang L, Liu J, Zhu Y, Wang F *et al*. Insights into the venom composition and evolution of an endoparasitoid wasp by combining proteomic and transcriptomic analyses. Sci Rep. 6: 19604, 2016.

Zhang C, Wang CZ. cDNA cloning and molecular characterization of a cysteine-rich gene from Campoletis chlorideae polydnavirus. DNA Sequence 14: 413-419, 2003.

Zhang G, Lu Z-Q, Jiang H, Asgari S. Negative regulation of prophenoloxidase (proPO) activation by a clip domain serine proteinase homolog (SPH) from endoparasitoid venom. Insect Biochem Mol. Biol. 34: 477-483, 2004.

Zhang G, Schmidt O, Asgari S. A calreticulin-like protein from endoparasitoid venom fluid is involved in host hemocyte inactivation. Dev. Comp. Immunol. 30: 756-764, 2006.

Zhu JY, Ye GY, Fang Q, Hu C. Alkaline phosphatase from venom of the endoparasitoid wasp, *Pteromalus puparum*. J. Insect Sci. 10: 14, 2010.