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***Nasonia vitripennis* venom causes targeted gene expression changes in its fly host**

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Abstract

Parasitoid wasps are diverse and ecologically important insects that use venom to modify their host's metabolism for the benefit of the parasitoid's offspring. Thus, the effects of venom can be considered an 'extended phenotype' of the wasp. The model parasitoid wasp *Nasonia vitripennis* has approximately 100 venom proteins, 23 of which do not have sequence similarity to known proteins. Envenomation by *N. vitripennis* has previously been shown to induce developmental arrest, selective apoptosis and alterations in lipid metabolism in flesh fly hosts. However, the full effects of *Nasonia* venom are still largely unknown. In this study, we used high throughput RNA sequencing (RNA-Seq) to characterize global changes in *Sarcophaga bullata* (Diptera) gene expression in response to envenomation by *N. vitripennis*. Surprisingly, we show that *Nasonia* venom targets a small subset of *S. bullata* loci, with ~2% genes being differentially expressed in response to envenomation. Strong upregulation of *enhancer of split* complex genes provides a potential molecular mechanism that could explain the observed neural cell death and developmental arrest in envenomated hosts. Significant increases in antimicrobial peptides and their corresponding regulatory genes provide evidence that venom could be selectively activating certain immune responses of the hosts. Further, we found differential expression of genes in several metabolic pathways, including glycolysis and gluconeogenesis that may be responsible for the decrease in pyruvate levels found in envenomated hosts. The targeting of *Nasonia* venom effects to a specific and limited set of genes provides insight into the interaction between the ectoparasitoid wasp and its host.

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Data accessibility

Raw reads from all samples are submitted to SRA study accession SRP044688. The transcriptome assembly is submitted to BioProject Database (<http://www.ncbi.nlm.nih.gov/bioproject/>) accession PRJNA255811.

Supporting information

Additional supporting information may be found in the online version of this article.

Keywords

enhancer of split; extended phenotype; parasitoid wasps; venom

Introduction

Parasitoid wasps are abundant and ecologically important organisms that inject venom into and lay their eggs in or on other insects (Godfray 1994; Quicke 1997). There are an estimated 150 000–600 000 species of parasitoid wasps that utilize a diverse array of host insect species and developmental stages (Quicke 1997; Heraty *et al.* 2011). Through their venom, these wasps are known to induce a variety of metabolic and immunologic manipulations of their host insects to provide resources for the development of the wasp's offspring (Rivers & Denlinger 1994, 1995b; Parkinson *et al.* 2001, 2002; Rivers *et al.* 2002a,b; Federici & Bigot 2003; Danneels *et al.* 2010; De Graaf *et al.* 2010). As such, the venom of parasitoids can be considered an 'extended phenotype' (Dawkins 1999) that manipulates the host's physiology to provide an environment more conducive for development of the wasp's offspring. Parasitoid wasps are either endoparasitic or ectoparasitic. Endoparasitoid wasps lay their eggs within the tissue of their hosts, and the primary effects of their venoms on hosts are temporary paralysis and immune suppression to protect the wasp's offspring from encapsulation and other negative immune responses (Parkinson *et al.* 2002; Vincent *et al.* 2010; Mortimer *et al.* 2013). Some endoparasitoid wasp species harbour polydnavirus symbionts that also alter the immune system and development of the parasitoid's host (Burke & Strand 2012). In contrast, ectoparasitoid wasps lay their eggs on the surface of their hosts, and their venoms primarily alter host development and metabolism, to provide an improved resource for their feeding offspring (Rivers & Denlinger 1994; Nakamatsu & Tanaka 2003). The venom of parasitoid wasps holds enormous potential as sources for new bioactive compounds and drug discovery (Danneels *et al.* 2010; De Graaf *et al.* 2010); however, the full extent of the venom on host physiology and the mechanisms behind their effects are still unknown.

Nasonia vitripennis (Hymenoptera: Pteromalidae) (hereafter *Nasonia*) is the genetic model for ectoparasitoid wasps, with a complete genome sequence and several powerful genetic tools available (Werren & Loehlin 2009a; Werren *et al.* 2010). *Nasonia* is a pupal ectoparasitoid and a generalist in terms of host preference, including the flesh fly *Sarcophaga bullata* (Diptera: Sarcophagidae) (Rivers & Denlinger 1995a). Because *Nasonia* lays its eggs between the host pupa and the puparium wall, eggs can be removed, thereby eliminating confounding effects of larval feeding and isolating the effects of envenomation. Nearly 100 venom proteins have been identified in *Nasonia*, 23 of which have no sequence similarity to known proteins (De Graaf *et al.* 2010; Werren *et al.* 2010). *Nasonia* whole venom induces developmental arrest of the host pupa (Rivers & Denlinger 1995b), depression in respiration (Rivers & Denlinger 1994), decrease in pyruvate metabolism (Rivers & Denlinger 1994), alteration in lipids (Rivers & Denlinger 1995b) and suppression of the host immune response (Rivers *et al.* 2002a,b). Little is known, however, about the specific mechanisms by which venoms induce these observed changes in the hosts or the full range of physiological and metabolic effects.

Most previous studies on *Nasonia* venom functions have focused on specific phenotypic effects, such as oxygen consumption, pyruvate levels or lipid composition (Rivers & Denlinger 1994, 1995b; Rivers *et al.* 2002a), or have been limited to a predetermined set of genes in a microarray (Danneels *et al.* 2013) or metabolites (Mrinalini *et al.* 2014). However, new sequencing technologies allow for the comparison of gene expression changes across the entire genome of envenomated and normally developing hosts. These data can reveal both small and large venom-mediated changes in gene expression of major metabolic pathways, including previously unknown venom effects on host physiology and metabolism, as well as facilitate inference of potential mechanisms behind known venom effects.

To determine the effects of *N. vitripennis* venom on gene expression in the host *S. bullata*, we used whole-body RNA sequencing (RNA-Seq) to sequence envenomated, normally developing and mechanically injured hosts at six time points ranging from 4 to 120 h post-envenomation. By comparing gene expression profiles within and between treatments as well as a global metabolic study of venom alteration in *S. bullata* (Mrinalini *et al.* 2014), we report (i) venom-induced changes in gene expression during *S. bullata* development, (ii) effects of simulated physical injury by *Nasonia*'s ovipositor and (iii) several possible mechanisms by which the venom initiates changes in host development, metabolism and immunity. Additionally, we report the *S. bullata* transcriptome assembly, thereby providing a valuable resource that will aid studies on important aspects of insect biology, such as diapause and immune stimulation.

Methods and materials

Sarcophaga bullata and *Nasonia* preparation

A colony of *S. bullata* is maintained as previously described (Werren & Loehlin 2009b). For each of the three replicates, we used pupae that had pupated within a 12-h window of time to standardize the age of the hosts. Pupae were allowed to develop for a further 3 days at 30 °C and then kept at room temperature at 25 °C (<10 h) until needed. Newly enclosed and mated female *Nasonia* (strain AsymCx) ($N=80$) were isolated in single test tubes containing two *S. bullata* pupa for 8 h to precondition stinging and host-feeding behaviours. After this period, *S. bullata* pupae were removed and the *Nasonia* were kept at 25 °C for 16 h without hosts to encourage stinging and oviposition during the experiment.

Experimental setup and protocol

The experiment is designed to identify the effects of *Nasonia* venom on gene expression in the host *S. bullata* by comparing envenomated, normally developing and mechanically injured hosts at 4, 8, 24, 36, 72 or 120 h after envenomation (Fig. 1). Three replicates for each treatment described below were performed on different dates (May, July and September 2012) to provide biological and technical replication. *Sarcophaga bullata* pupae ($N=240$) were placed in drilled foam plugs leaving only the anterior portion of the puparium exposed for *Nasonia* stinging and oviposition, the anterior portion was chosen for ease of parasitoid egg removal. Prepared host pupae were randomly and equally divided into three experimental treatment groups: envenomated, mechanically injured and normally developing. For the envenomation treatment group, hosts were placed in tubes containing

preconditioned *Nasonia* and kept at 25 °C for 4 h, allowing wasps sufficient time for stinging. Mechanically injured treatment group involved hosts punctured with an insect pin (size 00) between the second and third puparial segments. This was used to simulate insertion of *Nasonia* ovipositor into hosts to differentiate the effects of mechanical injury from the effects of envenomation. Normally developing and mechanically injured hosts were treated under identical conditions as envenomated hosts, with the exception that no *Nasonia* was added to the hosting tube.

All hosts were removed from their plugs after 4 h, and 2–3 anterior segments of the pupal case were carefully removed to keep the host pupa intact within the puparium. Hosts were examined to confirm stinging or mechanical damage, both evidenced by a melanization reaction at the site. *Nasonia* eggs laid on envenomated hosts were removed using a paintbrush. Hosts that did not have a sting site and eggs were excluded from the study. Transparent gel caps (Medisa Inc.) were used to protect the anterior of host puparia from desiccation and injury. The processed hosts were kept at 25 °C until collection time. For each treatment, hosts were divided into six time points: collected at 4, 8, 24, 36, 72 or 120 h after the pupae were first placed into hosting tubes. For each time point, six *S. bullata* per treatment ($N=126$) were collected for RNA extraction. Gel caps were removed, and each pupa was removed using fine-tipped forceps and placed into tubes containing 1 mL of TRIzol® (Life Technologies). Hosts were homogenized using plastic pestles, and the resulting homogenates were stored at –80 °C until all time points from a replicate set were processed.

Total RNA extraction and sequencing

Extraction of total RNA was carried out in TRIzol® following manufacturer's instructions (Life Technologies). Basic quantification and quality assessments were performed using a NanoDrop ND-1000 Spectrophotometer (ThermoScientific). The five highest quality RNA extractions for each treatment and time point were then pooled ensuring equimolarity of each sample in a final 20 µL volume at a total RNA concentration of 1 µg/µL. Three replicates of the five pooled RNA samples were submitted to the Epigenomics Core Facility of Weill Cornell Medical College for cDNA library preparation and sequencing by 50 bp single-end read Truseq RNA sequencing on an Illumina HiSeq 2000 machine.

Sarcophaga bullata reference transcriptome

To ensure a high-quality reference transcriptome for mapping, we did additional sequencing of envenomated and normally developing hosts collected over the same six time periods with 454 sequencing. A normalized library of all samples pooled was sequenced using GS FLX titanium reagents on a full 454 sequencing plate at the Indiana University Sequencing Core, Bloomington, Indiana, USA.

Reads obtained from the 454 plate were quality-trimmed to a minimum Phred score of 30 using SolexaQA v2.1 (Cox *et al.* 2010). Reads shorter than 50 base pairs (bp) were removed using prinseq-lite (version 0.20.3) '-min_len' switch (Schmieder & Edwards 2011). Similarly, the Illumina reads were quality-trimmed to a minimum Phred score of 40 across the entire sequence and reads smaller than 20 bp or containing any Ns were removed. To

reduce memory requirements and effects of PCR artefacts on the assembly, exact duplicate Illumina reads were removed using the derep switch in prinseq-lite. A transcriptome assembly of both 454 and Illumina reads was carried out with Oases v0.2.08 using the packages oases_pipeline python script with Kmer ranges 12–39 and coverage set to 6× (Schulz *et al.* 2012).

The longest transcript from each oases locus was selected to represent that locus, to reduce sequence redundancy generated by transcript isoforms. To ensure that high-expressing, conserved genes were not erroneously removed in this step, previously removed contigs with good BLAST homology to *Drosophila melanogaster* transcripts (e-value <1e-50) and high expression (RPKM > 100) were added back to the data set. Next, a mapping scaffold was generated using an in-house script to join the transcripts using 150 N spacer sequences, as well as to generate a gene feature file that described the position of each transcript in the scaffold. To filter out loci that had very low expression, all of the good quality Illumina reads were mapped to the reference and counts were generated using HTSeq-count with the intersection-strict mode (Anders *et al.* 2014). Any transcripts with <1 count per million input reads were removed. The reference transcriptome was annotated using a BLASTX search of the nr database (e-value <1e-5). Additional local databases from the published genomes of several model organisms (*Anopheles gambiae*, *Drosophila melanogaster*, *Xenopus laevis*, *Mus musculus*, *Homo sapiens*, *Rattus norvegicus* and *Danio rerio*) were also searched using BLASTX to evaluate the consistency of the annotation across model organisms. Gene ontology (GO) terms were assigned by BLAST2GO v2.5.0 (Conesa *et al.* 2005).

Data analyses

Tophat2 v2.0.4 (default settings) was used to map Illumina reads to the reference transcriptome for fragments per kilobase per million (FPKM) values, and counts for each condition and replicate were generated using HTSeq-count with the intersection-strict mode (Kim *et al.* 2013). DESeq v1.12.1 was used to generate normalized read counts and differential expression calls for each time point and treatment (Anders & Huber 2010). In all comparisons, three biological replicates of each treatment group were used to calculate differential expression. Clustering was performed on temporal expression within treatment using the k-means analysis in JMP Pro®, v10.0.0 (SAS Institute Inc). A multidimensional scaling analysis and global linear analysis of all replicates were performed in EdgeR (Robinson *et al.* 2010; McCarthy *et al.* 2012).

Three comparisons were made to determine groups of differentially expressed genes among treatments (Fig. 1): (i) to determine the effect of physical injury by the ovipositor on *S. bullata*, we compared normally developing and mechanically injured hosts during the first 24 h when almost all differential gene expression occurred; (ii) to determine temporal patterns of gene expression within normally developing and envenomated hosts, we compared individual time points within treatments to the 4-h time point; and (iii) to determine the differences between normally developing and envenomated hosts, we directly compared envenomated to normally developing hosts at the same time point. To be considered significantly differentially expression, a contig needed an adjusted *P*-value <0.05 with DESeq and a false discovery rate (FDR) adjusted *P*-value <0.05 with EdgeR GLM. Only

contigs that were significantly differentially expressed in both tests were included in further analyses. Significantly, overrepresented GO categories were determined using BINGO in Cytoscape with a adjusted P -value <0.01 (Maere *et al.* 2005).

Results and discussion

Sarcophaga bullata reference transcriptome

We generated a de novo *S. bullata* pupal reference transcriptome assembly in Oases using a combination of high-quality 454 (1 357 655 reads) and Illumina (37 767 128 reads) sequencing data (see Methods). The raw assembly consisted of 113 689 transcripts with an average length of 1427 bp (Table 1). Filtering (see Methods and Table 1) resulted in a final set of 8234 contigs that we refer to as the 'core group' in the following analyses.

The *S. bullata* reference transcriptome was assessed for completeness using BLASTX to the NCBI protein database. Among the 8234 core transcripts, 6653 (80%) had a hit, with dipteran species making up the majority of top hits. We assessed the level of transcript fragmentation in the core group by analysing BLASTX alignments between *D. melanogaster* proteins and *S. bullata*-translated transcripts. On average, 73% of each *D. melanogaster* protein aligned with a *S. bullata* sequence, suggesting that the assembly was able to capture most of the coding region. Additionally, the *S. bullata* assembly recovered 87% of the highly conserved pupal-expressed transcripts from *D. melanogaster* with reciprocal BLAST, indicating that the transcriptome is likely to be a good quality representation of the pupal life stage of *S. bullata*. Interestingly, 79 of the *S. bullata* transcripts lacked a clear BLASTX homologue in Drosophilidae and yet had other hits in NCBI. Most of these sequences (62%) have a top hit to uncharacterized and hypothetical proteins. The two major groups of annotated proteins had hits to hexamerins and transposable elements. The hexamerins are a conserved group of proteins that predominately act as storage proteins in insects. Previous studies in the brachyceran Diptera have shown significant turnover within this group (Burmester *et al.* 1998). Finally, we compared the *S. bullata* transcriptome to the recently completed transcriptome of the closely related *Sarcophaga crassipalpis* (Hahn et al. 2009). BLASTN comparisons showed good overlap among the *S. bullata* core group transcripts, with 89% having a strong hit (bit score >100) to one of 6451 *S. crassipalpis* sequences. The combination of 454 and Illumina data helped create a longer assembly for *S. bullata*, with the average query length in *S. bullata* being 1963 bp (N50 2480 bp) compared to the 454-only assembly of *S. crassipalpis* with an average of 331 bp (N50 321 bp).

Summary of normally developing and envenomated host gene expression

Reads from envenomated, normally developing and mechanically injured treatments were mapped to 8234 genes in the *S. bullata* reference transcriptome, with a range of FPKM values from 0 to 375 798 (average 273.0 RPKM). Among the 18 samples sequenced (six time points by three treatments), only 17.2% (1418/8234) of the genes differed significantly in their expression either within or between treatments, leaving 82.8% of the genes with a relatively constant expression level across all samples (Fig. S1, Supporting information).

A multidimensional scaling analysis on the expression profile of all genes shows no universal clustering by treatment, collection time or replicate (Fig. 2); however, several clusters did emerge. All envenomated hosts from 24 to 120 h form a cluster, demonstrating that there is a unique gene expression profile in later envenomated hosts that is distinct from normally developing hosts. Additionally, normally developing hosts at 72 and 120 h formed a distinct cluster, indicating an abrupt shift in pupal development between 36 and 72 h. The lack of distinct groups in earlier time points (envenomated hosts from 4 to 8 h and normally developing hosts from 4 to 36 h) can be explained by the low level of significantly differentially expressed genes between envenomated and normally developing hosts collected before the 72-h time point (described below).

Twenty *Nasonia* genes were identified by BLASTX in one 72-h and two 120-h envenomated replicates. Despite this contamination, we found no significant changes in *S. bullata* expression in these samples compared to replicates at the same time point without *Nasonia* genes (Fig. S2, Supporting information). This observation suggests that only a small number of *Nasonia* larvae escaped detection and their limited feeding had little effect on the host at the molecular level.

Effects of mechanical injury

To determine whether the effects of mechanical injury from the wasp's ovipositor induce any part of the envenomated phenotype, we compared the gene expression of hosts that were mechanically injured (with an insect pin) to that of normally developing hosts (Fig. 1a). Mechanically injured hosts had a quick and limited response compared to envenomated hosts, with only 20 differentially expressed genes at 4 h, three at 8 h and one at 24 h out of 8234 *S. bullata* genes (Table S1, Supporting information). Overrepresented GO terms in this group include response to stimulus, immune response and response to wounding. The genes with the largest response were three attacin genes (A, B, and D), which are antibacterial genes in *D. melanogaster*, and the gene *pale*, which is a direct target of the wound-induced signal transduction pathway involved in cuticular wound repair (Mace *et al.* 2005).

Whereas none of the mechanically injured differentially expressed genes overlapped with envenomated genes at a given time point, five of the 4-h genes were shared at later envenomated time points (i.e haemolectin, attacin and cytochrome *c*). However, envenomation had a larger effect upon these genes with an average upregulated fold change of 14.1 compared to 2.7 in the mechanically injured hosts. The small amount of overlap in differentially expressed genes, the difference in expression amplitude and the differences in timing of the response all indicate that the physical wounding of the host by *Nasonia*'s ovipositor plays only a trivial role in the venom response.

Temporal changes in genes expression within normally developing hosts

To determine gene expression changes throughout the development of normally developing and envenomated hosts, we compared the starting gene expression at 4 h to each subsequent time point in each treatment (Fig. 1b). From a total of 8234 *S. bullata* genes, normally developing hosts had 852 genes that were differentially expressed across time (Fig. 3b). Within the first 36 h, normally developing hosts had minimal gene expression change

(average 1.0 differentially expressed gene per time point) (Fig. 3a). Strikingly, the number of differentially expressed genes increased to an average of 668 genes at 72 and 120 h, indicating a developmental shift somewhere between 36 and 72 h. Genes that make up this developmental shift are identified and discussed in the developmental arrest section below.

Temporal changes in genes expression within envenomated hosts

Surprisingly, only 2.9% of total genes in the *S. bullata* transcriptome changed temporally in envenomated hosts. Envenomated hosts had a total of 244 differentially expressed genes, 164 genes were only differentially expressed in envenomated hosts, and 80 genes were differentially expressed in both envenomated and normally developing hosts (Fig. 3b). In contrast with normally developing hosts, envenomated hosts maintained a relatively constant level of differentially expressed genes across all time points (average 99.8 genes at any time point). They lacked the sudden increase in expression shown in their normally developing counterparts, with an increase in only 83 genes between 36 and 72 h (Fig. 3a). After envenomation, the vast majority of gene expression levels remain constant relative to the expression profile for when the host was initially stung, as no significant changes in expression can be seen in 97.1% of host genes from when the host was stung to any subsequent time point. This suggests that venom directly affects a relatively small subset of genes that change temporally in envenomated hosts. Several significant differences found in metabolites from a global metabolomics study of envenomated hosts were not mirrored in this transcriptome study (Mrinalini *et al.* 2014), suggesting that venom may be manipulating proteins and metabolites directly (e.g., via protein–protein interactions), as opposed to transcriptionally regulating pathways, to modify metabolism. Alternatively, by sequencing the transcriptome of whole pupa, changes in tissue-specific gene expression may not be detectable.

Comparing envenomated to normally developing host gene expression

The direct comparison of envenomated hosts to the corresponding normally developing host time points was used to look at the net effect of *Nasonia* venom on *S. bullata*. Overall, we found 1217 genes significantly differentially expressed between normally developing and envenomated hosts. Among all differentially expressed genes between treatments, 610 of these were also differentially expressed in temporal comparisons among normally developing hosts but not in envenomated hosts (Fig. 3b). These could be identified as genes most likely involved in the developmental arrest of the host. Within this set, a large subset showed sudden and dramatic expression change between 36 and 72 h (Fig. S3b, Supporting information). A set of 147 differentially expressed genes between treatments were shared with the temporal comparisons in envenomated hosts and identified as genes affected by venom, and 58 genes were shared in the temporal comparisons of both envenomated and normally developing hosts (Fig. 3b). Additionally, there were 402 genes that were not found in the within-treatment comparisons (Fig. 3b). These may represent genes that have small expression changes in opposite directions in envenomated and normally developing hosts, so that they are not significant within a treatment but become so when compared between treatments.

The general pattern shows that up to 36 h post-envenomation, there are a relatively small number of changes in gene expression and most are upregulated in envenomated hosts. After 36 h, there was a dramatic increase in the number of differentially expressed genes, most of which are associated with normal development genes that were not upregulated in envenomated hosts (Fig. S3b, Supporting information). Earlier time points (4–36 h) averaged 93.8 significantly differentially expressed genes per time point with 86.9% upregulated in envenomated hosts. However, later time points (72 and 120 h) had significantly more differentially expressed genes (average 867.5 genes per time point) with only 46.3% upregulated in envenomated hosts (Fig. S3b, Supporting information).

Whereas the majority of the genes had some functional assignment, a subset (~30%) remained completely uncharacterized. This subset was relatively consistently represented across all treatments and time points and did not show any abnormally large expression changes; therefore, the current annotations have probably captured most of the large patterns involved in *S. bullata* envenomation. The majority of genes with a functional annotation that were differentially expressed genes between envenomated and normally developing hosts fell into three broad categories: immunity, metabolism and developmental arrest.

Developmental arrest

The most striking phenotypic alteration of the envenomated host is its developmental arrest, characterized by lack of eye pigment deposition and bristle formation, lack of melanization in the cuticle and decreased oxygen consumption (Rivers & Denlinger 1994). Whereas developmental arrest induced by venom may superficially resemble pupal diapause, venom-induced arrest cannot be rescued by exogenous ecdysteroids (Rivers & Denlinger 1994) and ultimately results in pupal death. Our study shows that this arrest can also be observed on a transcriptional level by the sudden upregulation of genes at 72 h in normally developing hosts that does not occur in envenomated flies (Fig. 2). When comparing envenomated to normally developing hosts, genes associated with developmental arrest increase from 9.9% of genes differentially expressed between 4 and 36 h to 54.8% between 72 and 120 h.

This dramatic increase of differentially expressed normally developing genes between 36 and 72 h contain genes with annotations that we would expect in pupal development with overrepresented GO terms of chitin metabolism, myofibril assembly, and electron transport chain (Table S2, Supporting information). In 72-h envenomated hosts, cuticular proteins had some of the largest decreases, with ten transcripts ranging from a 100- to 2072-fold decrease compared to normally developing flies. Other chitin genes also experienced significantly decreased expression such as chitin deacetylase (max 2.3 fold decrease) and chitin-binding domain protein peritrophin-A (max 568.7-fold decrease). Genes involved in myofibril assembly (some with specificity to flight muscles) such as actin (max 47.8-fold decrease), flightin (max 7.2-fold decrease) and myosin light chain 2 (max 5.1-fold decrease) did not increase in expression in envenomated hosts. Finally, expression levels of genes involved in energy production via the electron transport chain and the TCA cycle, such as electron transfer flavoprotein (2.5-fold decrease), NADH dehydrogenase (2.7-fold decrease), succinate dehydrogenase (2.0-fold decrease) and aconitase (2.3-fold decrease), significantly decreased in envenomated hosts. The expression of these genes was arrested in envenomated

hosts and maintained at constant expression levels similar to 4–36 h normally developing hosts.

Possible mechanisms of developmental arrest

The sudden upregulation of >600 genes at 72 h in normally developing hosts (Fig. 3a), which is an attribute of pupal development, was not mirrored in envenomated hosts. In envenomated hosts, the expression levels of these dramatically changing pupal genes remained similar to expression levels in normally developing hosts between 4 and 36 h. This suggests that the shift in gene expression associated with normal development is not triggered in envenomated hosts; therefore, envenomated hosts may simply maintain *status quo* gene expression levels. Rivers *et al.* (2011) found that envenomated hosts have significant and selective apoptosis of brain tissue relative to fat body tissue 24 h poststinging. The targeted destruction of brain tissue could interfere with hormones that trigger metamorphosis, specifically prothoracicotropic hormone (PTTH), which is produced in the insect brain and is essential for the secretion of ecdysteroids. Changes in ecdysteroid titres in insect pupae have been shown to initiate dramatic cascades in gene transcription (Andres *et al.* 1993). With selective damage to the neural tissue, *Nasonia* venom could induce developmental arrest and redirect the physiology of the fly for the benefit of its progeny (Rivers *et al.* 2011).

A family of significantly upregulated genes, the *enhancer of split* complex (*e(spl)*-C), may be involved in directed apoptosis of neural tissue and subsequent developmental arrest (Fig. 4). The *e(spl)*-C members are partially redundant genes that encode transcription factors of the basic helix-loop-helix (bHLH) family and are known to suppress neurogenesis (Nakao & Campos-Ortega 1996). Proneural cells that retain high expression of *e(spl)*-C genes either undergo programmed cell death or differentiate into epidermal cells, whereas proneural cells with low *e(spl)*-C expression develop into neural cells. Persistent expression of *e(spl)*-C genes in *D. melanogaster* causes severe neural and imaginal sensory organ defects by apoptosis in proneural cells (Nakao & Campos-Ortega 1996). Whereas the exact mechanism of how *e(spl)*-C genes induce apoptosis in cells is not known, it is known that DNA binding is not essential (Nakao & Campos-Ortega 1996). All *e(spl)*-C genes present in our transcriptome were significantly upregulated in envenomated hosts in at least one time point sampled (4–120 h). Proteins *m1*, *m2*, *m4*, *m5*, *m7*, *m8*, *malpha*, *mbeta* and *mdelta* from the *e(spl)*-C were significantly upregulated ranging from a 2.6- to 6.5-fold increase, with their expression peaking at 24 or 36 h post-envenomation (Fig. 4).

The *e(spl)*-C is part of the notch signalling pathway, which is a key pathway involved in the control of multiple cell differentiation processes during embryonic and adult life in metazoans (Guruharsha *et al.* 2012). To initiate the *e(spl)*-C, a notch protein is cleaved by a repolysin-like metalloproteinase to release a Notch intracellular domain (NICD), which binds to Suppressor of Hairless (*SU(H)*) and subsequently induces the transcription of *e(spl)*-C, which typically inactivates the expression of multiple developmental genes. Interestingly, a metalloproteinase has also been found in *Nasonia* venom (Danneels *et al.* 2010; De Graaf *et al.* 2010). The top sequence hit to this protein outside of *Nasonia*, is to the venom gland-expressed metalloproteinase of the parasitic wasp *Eulophus pennicornis* (e-

value = $3e^{-45}$). When recombinant *E. pennicornis* metalloproteinases were injected into fifth instar larvae of their host, *Lacanobia oleracea*, partial mortality was observed due to unsuccessful moulting, and surviving individuals showed significantly slowed development and growth (Price *et al.* 2009). Furthermore, injected larvae seemed to miss a developmental cue by continuing to gain weight, while normal larvae lost weight in preparation for pupation. Interference in the notch signalling pathway was therefore suggested as a possible mechanism for *E. pennicornis* (Price *et al.* 2009), and our results indicate that this may be shared with *Nasonia*.

Defence and stress genes

It has previously been reported that within an hour of envenomation, the number of circulating hemocytes (cells involved in insect immunity and found in haemolymph) is severely reduced (Rivers *et al.* 2002b; Zhang *et al.* 2005). Other immune responses, such as melanization and clotting of host haemolymph, are also suppressed in envenomated flies (Rivers *et al.* 2002a). We found no evidence of immune suppression in the host transcriptome, which may suggest that the previously observed immune responses were the result of protein–protein interactions and not transcriptionally regulated. In contrast, we found a large upregulation of the coagulation gene haemolysin (50 fold increase at 36 h) in envenomated hosts, which may be a compensatory response of *S. bullata* to the venom's suppression of haemolymph clotting. Additionally, the plasmatocyte surface protein, Nimrod (max 39× fold increase) (Kurucz *et al.* 2007), and a mitogen-activated protein kinase (max 39× fold increase), which have been shown to regulate hemocyte production (Zettervall *et al.* 2004), were also upregulated. These responses do not seem to restore the circulating hemocyte number or clotting response, as neither recover in envenomated hosts (Rivers *et al.* 2002a). Unsurprisingly, several heat-shock proteins were upregulated in the envenomated host. Heat-shock proteins are highly expressed when organisms are exposed to elevated temperatures or other stresses, and are involved in the folding and unfolding of other proteins. Heat-shock proteins 27 (85×), 70 (77×), 23 (21×) and 83 (5.3×) all showed significantly increased expression in response to envenomation.

It would be expected that envenomation would repress the host's immune response to allow the wasp offspring to feed more readily; however, this may also expose the host to bacterial or fungal infections that would diminish its quality. With this in mind, it is interesting that several genes that encode antimicrobial peptides, which are specific to suppression of bacteria and fungi infection, were significantly upregulated following *Nasonia* envenomation. Cericropin (3.4×), dipterocin (4.7×), attacin (5.1×) and sapecin (or defensin) (1.76×) showed the largest expression changes. Genes in both the toll and immune deficiency (IMD) pathways that regulate antimicrobials are also upregulated (Lemaitre *et al.* 1997). In the toll pathway, *spätzle* (8.6×) and *cactus* (1.8×) are upregulated; however, *toll* itself is slightly downregulated (−3.3-fold change, though not statistically significant). In the IMD pathway, *relish* (2.4×) is upregulated. Interestingly, when we examined the gene expression from the *Nasonia* larvae that were failed to be removed in some replicates, some of the highest expressing genes at 72 and 120 h in the *Nasonia* larvae were also genes encoding antimicrobial peptides. This included nahymenopteracecin, nasonin, abaecin, clausin and apolipophorin.

Whether *Nasonia* venom has a direct effect on the activation of the toll and IMD pathways is not known. It has been proposed that serine proteases present in the venom could process *spätzle*, thereby directly activating the toll pathway (Danneels *et al.* 2010) as a preventative measure against bacterial or fungal infection. However, it is also possible that the venom simply leaves the antimicrobial pathways unaltered and they are activated after microbes invade immune-suppressed hosts. These alternatives have not been resolved.

Metabolism

One of the more striking results from our study is that *Nasonia* venom seems to affect a relatively small number of temporally changing genes in envenomated hosts (244 of 8234). The vast majority of genes stay at a relatively consistent expression level that is similar with levels observed in 4–36-hr normally developing hosts. Among genes that show expression changes, most deal with stress and defence responses, whereas metabolic genes remain stable with two exceptions: tRNA synthetases and pyruvate metabolism. Glutaminyl-, lysyl-, valyl-, tyrosyl-, tryptophanyl- and aspartyl-tRNA synthetases were all significantly upregulated in envenomated hosts, and a further 10 of the 33 total tRNA synthetases were also upregulated, although not significantly. An increase in free amino acids in envenomated hosts (Mrinalini *et al.* 2014) may explain the upregulation in nearly half the tRNA synthetases present in the *S. bullata* transcriptome, which have the primary function of binding specific amino acids onto their appropriate tRNA. However, tRNA synthetases can be multifunctional proteins regulated by a diverse set of control mechanisms. For example, in humans, glutaminyl-tRNA synthetase can block apoptosis induced by heat shock (Ko *et al.* 2001). It is therefore possible that these tRNA synthetases are performing a yet unknown function in envenomated hosts.

Pyruvate metabolism

Changes in the regulation of multiple enzymes involved in pyruvate metabolism were found that are consistent with significant decreases in pyruvate levels in envenomated hosts found in previous studies (Rivers & Denlinger 1994; Mrinalini *et al.* 2014). Pyruvate is essential in several important metabolic pathways; it is synthesized via glycolysis and provides energy for both aerobic and anaerobic respiration (Fig. 5). It has been previously shown that oxygen consumption of the host is drastically reduced (>85%) soon after envenomation causing the host to function in a hypoxic state (Rivers & Denlinger 1994). The current study shows a significant upregulation of lactate dehydrogenase (max 16-fold increase) in envenomated hosts from 24 to 120 h, which converts pyruvate to lactic acid under anaerobic conditions. Figure 5 compares expression data to a prior metabolic analysis for pyruvate metabolism. Alterations were also found to other pathways that could be contributing to the depletion of pyruvate. Specifically, malic enzyme, which converts pyruvate into malate, was upregulated (max 10.3-fold increase) from 24 to 120 h and could indicate increased mobilization of pyruvate to form malate (Fig. 5). This mode of pyruvate depletion is further supported by the significant increase in malate in 72- and 120-h envenomated hosts (Mrinalini *et al.* 2014). This reaction could also explain the increase in malate in contrast to the decrease in most other TCA cycle metabolites and provide further support that venom could be interfering with oxidative metabolism (Mrinalini *et al.* 2014).

Pyruvate levels could further diminish by its conversion to glucose via the gluconeogenesis pathway. Although genes shared between gluconeogenesis and glycolysis do not show any significant changes in expression, two genes unique to gluconeogenesis, pyruvate carboxylase (max 8.2-fold) and phosphoenolpyruvate carboxykinase (max 15-fold) are both significantly upregulated from 24 to 120 h in envenomated hosts (Fig. 5). These two enzymes act together to reverse the final step of glycolysis performed by pyruvate kinase, thereby initiating gluconeogenesis. Direct observation of significantly lower levels of pyruvate support this; however, glucose levels do not change between envenomated hosts and normally developing hosts (Mrinalini *et al.* 2014), which would be expected if gluconeogenesis were upregulated. Mrinalini *et al.* (2014) also found significant increases in sorbitol in envenomated hosts, suggesting that excess glucose could be converted into sorbitol via increased aldose reductase activity in the sorbitol pathway. A gene with the annotation of aldose reductase is upregulated (max 5.6 fold at 36 h) in envenomated hosts. Increases in sorbitol have also been recorded in diapause and temperature-stressed insects (Chino 1958; Salvucci 2000).

The current study has several parallels with a microarray study conducted on another *Nasonia* host, *S. crassipalpis* (Danneels *et al.* 2013). Similar lists of overrepresented GO terms were identified during normal fly development. In both host species, *Nasonia* envenomation significantly increases lactate dehydrogenase and upregulated heat-shock proteins and other defensive genes. Sequences from the enhancer of split complex were not included in the microarray, so the developmental arrest hypothesis presented in this study could not be compared to the microarray study in *S. crassipalpis*. Our study identified additional pathways potentially depleting pyruvate (malic enzyme and gluconeogenesis) and increased antimicrobial peptide expression (e.g. cercropin and dipterin) that were not seen in *S. crassipalpis*. Differences between the studies could be accounted for by different sample times (3–24 h vs. 4–120 h), host species and preparation, and methods used to detect gene expression changes (microarray vs. de novo transcriptomes).

Conclusions

We ascertained genomewide alteration in host gene expression induced by *Nasonia* venom through the first 5 days of the envenomated phenotype. While envenomation causes large morphological effects, only a very small subset of genes (~2%) showed significant changes in their expression levels. From this small set of genes, we were able to generate a series of hypotheses for the mechanics driving different venom effects for further testing. Developmental arrest, which is the most striking phenotypic change, could clearly be observed at the transcriptional level. The venom-mediated mechanism that induces developmental arrest may involve the upregulation of the *enhancer of split* complex. *Nasonia* venom induces upregulation of antimicrobial peptides, which could be involved in protecting the host from microbial growth that could diminish the nutritional quality of the host for the wasp larvae. Furthermore, our results show that venom changes *S. bullata* metabolism by manipulating genes in multiple pathways leading to depletion in pyruvate levels. The profile of global transcriptional changes induced by venom of the parasitoid model *Nasonia* documented here is a first step towards putting venom function and evolution into an ecological context. *Nasonia* is a generalist parasitoid of flies from different families (e.g.

Sarcophagidae, Calliphoridae, Muscidae), whereas its close congeners *N. giraulti*, *N. longicornis* and *N. oneida* specialize on Protocalliphora blowflies occurring in bird nests. Future research will explore how host use influences the venom repertoire of these parasitoids and to what extent physiological effects of venoms generalize across fly species, for example does venom of the generalist *Nasonia* use the same mechanisms in all host species or do different host species have unique responses to envenomation. Additionally, we will be able to test how universal these mechanisms are in closely related species, such as *Nasonia giraulti*, a specialist on a single host species, whose venom composition varies from *N. vitripennis* (J. H. Werren, unpublished). If the mechanisms for developmental arrest or metabolic changes are universal, this may be useful in the biological control of pest insects, in which parasitoid wasps are already used. Furthermore, the limited and specific targeting of *Nasonia* venom to a controlled set of genes supports the potential of *Nasonia* venom as a source of highly specific bioactive molecules, with potential pharmaceutical value. Future knockdowns of individual venom proteins can provide a finer scale and an improved mechanistic understanding of individual venom functions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

- Anders S, Huber W. Differential expression analysis for sequence count data. *Genome Biology*. 2010; 11:R106. [PubMed: 20979621]
- Anders S, Pyl PT, Huber W. HTSeq—A Python framework to work with high-throughput sequencing data. *Bioinformatics*. 2014; doi: 10.1093/bioinformatics/btu638
- Andres AJ, Fletcher JC, Karim FD, Thummel CS. Molecular analysis of the initiation of insect metamorphosis: a comparative study of *Drosophila* ecdysteroid-regulated transcription. *Developmental Biology*. 1993; 160:388–404. [PubMed: 8253272]
- Burke GR, Strand MR. Polydnviruses of parasitic wasps: domestication of viruses to act as gene delivery vectors. *Insects*. 2012; 3:91–119. [PubMed: 26467950]
- Burmester T, Massey HC Jr, Zakharkin SO, Benes H. The evolution of hexamerins and the phylogeny of insects. *Journal of Molecular Evolution*. 1998; 47:93–108. [PubMed: 9664700]
- Chino H. Carbohydrate metabolism in the diapause egg of the silkworm, *Bombyx mori*—II: conversion of glycogen into sorbitol and glycerol during diapause. *Journal of Insect Physiology*. 1958; 2:1–12.
- Conesa A, Götz S, García-Gómez JM, et al. Blast2GO: a universal tool for annotation, visualization and analysis in functional genomics research. *Bioinformatics*. 2005; 21:3674–3676. [PubMed: 16081474]
- Cox MP, Peterson DA, Biggs PJ. SolexaQA: at-a-glance quality assessment of Illumina second-generation sequencing data. *BMC Bioinformatics*. 2010; 11:485–491. [PubMed: 20875133]
- Danneels EL, Rivers DB, De Graaf DC. Venom proteins of the parasitoid wasp *Nasonia vitripennis*: recent discovery of an untapped pharmacopee. *Toxins*. 2010; 2:494–516. [PubMed: 22069597]

- Danneels EL, Formesyn EM, Hahn DA, et al. Early changes in the pupal transcriptome of the flesh fly *Sarcophaga crassipalpis* to parasitization by the ectoparasitic wasp, *Nasonia vitripennis*. *Insect Biochemistry and Molecular Biology*. 2013; 43:1189–1200. [PubMed: 24161520]
- Dawkins, R. *The Extended Phenotype: The Long Reach of the Gene*. Oxford University Press; Oxford: 1999.
- De Graaf DC, Aerts M, Brunain M, et al. Insights into the venom composition of the ectoparasitoid wasp *Nasonia vitripennis* from bioinformatic and proteomic studies. *Insect Molecular Biology*. 2010; 19:11–26. [PubMed: 20167014]
- Federici B, Bigot Y. Origin and evolution of polydnaviruses by symbiogenesis of insect DNA viruses in endoparasitic wasps. *Journal of Insect Physiology*. 2003; 49:419–432. [PubMed: 12770621]
- Godfray, HCJ. *Parasitoids: Behavioral and Evolutionary Ecology*. Princeton University Press; Princeton, New Jersey: 1994.
- Gururharsha K, Kankel MW, Artavanis-Tsakonas S. The Notch signalling system: recent insights into the complexity of a conserved pathway. *Nature Reviews Genetics*. 2012; 13:654–666.
- Hahn DA, Ragland GJ, Shoemaker DD, Denlinger DL. Gene discovery using massively parallel pyrosequencing to develop ESTs for the flesh fly *Sarcophaga crassipalpis*. *BMC Genomics*. 2009; 10:234. [PubMed: 19454017]
- Heraty J, Ronquist F, Carpenter JM, et al. Evolution of the hymenopteran megaradiation. *Molecular Phylogenetics and Evolution*. 2011; 60:73–88. [PubMed: 21540117]
- Kim D, Pertea G, Trapnell C, et al. TopHat2: accurate alignment of transcriptomes in the presence of insertions, deletions and gene fusions. *Genome Biology*. 2013; 14:R36. [PubMed: 23618408]
- Ko Y-G, Kim E-K, Kim T, et al. Glutamine-dependent antiapoptotic interaction of human glutamyl-tRNA synthetase with apoptosis signal-regulating kinase 1. *Journal of Biological Chemistry*. 2001; 276:6030–6036. [PubMed: 11096076]
- Kurucz E, Márkus R, Zsámboki J, et al. Nimrod, a putative phagocytosis receptor with EGF repeats in *Drosophila* plasmatocytes. *Current Biology*. 2007; 17:649–654. [PubMed: 17363253]
- Lemaitre B, Reichhart J-M, Hoffmann JA. *Drosophila* host defense: differential induction of antimicrobial peptide genes after infection by various classes of microorganisms. *Proceedings of the National Academy of Sciences*. 1997; 94:14614–14619.
- Mace KA, Pearson JC, McGinnis W. An epidermal barrier wound repair pathway in *Drosophila* is mediated by grainy head. *Science*. 2005; 308:381–385. [PubMed: 15831751]
- Maere S, Heymans K, Kuiper M. BiNGO: a Cytoscape plugin to assess overrepresentation of gene ontology categories in biological networks. *Bioinformatics*. 2005; 21:3448–3449. [PubMed: 15972284]
- McCarthy DJ, Chen Y, Smyth GK. Differential expression analysis of multifactor RNA-Seq experiments with respect to biological variation. *Nucleic Acids Research*. 2012; 40:4288–4297. [PubMed: 22287627]
- Mortimer NT, Goecks J, Kacsoh BZ, et al. Parasitoid wasp venom SERCA regulates *Drosophila* calcium levels and inhibits cellular immunity. *Proceedings of the National Academy of Sciences*. 2013; 110:9427–9432.
- Mrinalini Siebert AL, Wright J, et al. Parasitoid venom induces metabolic cascades in fly hosts. *Metabolomics*. 2014; doi: 10.1007/s11306-014-0697-z
- 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. *Journal of Insect Physiology*. 2003; 49:149–159. [PubMed: 12770008]
- Nakao K, Campos-Ortega JA. Persistent expression of genes of the *Enhancer of Split* complex suppresses neural development in *Drosophila*. *Neuron*. 1996; 16:275–286. [PubMed: 8789943]
- 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 Biochemistry and Molecular Biology*. 2001; 31:57–63. [PubMed: 11102835]
- Parkinson N, Richards E, Conyers C, Smith I, Edwards J. Analysis of venom constituents from the parasitoid wasp *Pimpla hypochondriaca* and cloning of a cDNA encoding a venom protein. *Insect Biochemistry and Molecular Biology*. 2002; 32:729–735. [PubMed: 12044489]

- Price D, Bell H, Hinchliffe G, et al. A venom metalloproteinase from the parasitic wasp *Eulophus pennicornis* is toxic towards its host, tomato moth (*Lacanobia oleracae*). *Insect Molecular Biology*. 2009; 18:195–202. [PubMed: 19320760]
- Quicke, DL. *Parasitic Wasps*. Chapman & Hall Ltd; London: 1997.
- Rivers DB, Denlinger DL. Redirection of metabolism in the flesh fly, *Sarcophaga bullata*, following envenomation by the ectoparasitoid *Nasonia vitripennis* and correlation of metabolic effects with the diapause status of the host. *Journal of Insect Physiology*. 1994; 40:207–215.
- Rivers DB, Denlinger DL. Fecundity and development of the ectoparasitic wasp *Nasonia vitripennis* are dependent on host quality. *Entomologia Experimentalis et Applicata*. 1995a; 76:15–24.
- Rivers DB, Denlinger DL. Venom-induced alterations in fly lipid metabolism and its impact on larval development of the ectoparasitoid *Nasonia vitripennis* (Walker) (Hymenoptera: Pteromalidae). *Journal of Invertebrate Pathology*. 1995b; 66:104–110.
- Rivers D, Ruggiero L, Hayes M. The ectoparasitic wasp *Nasonia vitripennis* (Walker)(Hymenoptera: Pteromalidae) differentially affects cells mediating the immune response of its flesh fly host, *Sarcophaga bullata* Parker (Diptera: Sarcophagidae). *Journal of Insect Physiology*. 2002a; 48:1053–1064. [PubMed: 12770028]
- Rivers DB, Rocco MM, Frayha AR. Venom from the ectoparasitic wasp *Nasonia vitripennis* increases Na⁺ influx and activates phospholipase C and phospholipase A2 dependent signal transduction pathways in cultured insect cells. *Toxicon*. 2002b; 40:9–21. [PubMed: 11602274]
- Rivers DB, Keefer DA, Ergin E, Uçkan F. Morphology and ultrastructure of brain tissue and fat body from the flesh fly, *Sarcophaga bullata* Parker (Diptera: Sarcophagidae), envenomated by the ectoparasitic Wasp *Nasonia vitripennis* (Walker) (Hymenoptera: Pteromalidae). *Psyche: A Journal of Entomology*. 2011; 2011doi: 10.1155/2011/520875
- Robinson MD, McCarthy DJ, Smyth GK. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics*. 2010; 26:139–140. [PubMed: 19910308]
- Salvucci ME. Sorbitol accumulation in whiteflies: evidence for a role in protecting proteins during heat stress. *Journal of Thermal Biology*. 2000; 25:353–361. [PubMed: 10838174]
- Schmieder R, Edwards R. Quality control and preprocessing of metagenomic datasets. *Bioinformatics*. 2011; 27:863–864. [PubMed: 21278185]
- Schulz MH, Zerbino DR, Vingron M, Birney E. Oases: robust *de novo* RNA-seq assembly across the dynamic range of expression levels. *Bioinformatics*. 2012; 28:1086–1092. [PubMed: 22368243]
- Vincent B, Kaeslin M, Roth T, et al. The venom composition of the parasitic wasp *Chelonus inanitus* resolved by combined expressed sequence tags analysis and proteomic approach. *BMC Genomics*. 2010; 11:693–708. [PubMed: 21138570]
- Werren JH, Loehlin DW. The parasitoid wasp *Nasonia*: an emerging model system with haploid male genetics. *Cold Spring Harbor protocols*. 2009a; 2009:1–10.
- Werren JH, Loehlin DW. Rearing *Sarcophaga bullata* fly hosts for *Nasonia* (parasitoid wasp). *Cold Spring Harbor protocols*. 2009b; 10:1–4.
- Werren JH, Richards S, Desjardins CA, et al. Functional and evolutionary insights from the genomes of three parasitoid *Nasonia* species. *Science*. 2010; 327:343–348. [PubMed: 20075255]
- Zettervall C-J, Anderl I, Williams MJ, et al. A directed screen for genes involved in *Drosophila* blood cell activation. *Proceedings of the National Academy of Sciences of the United States of America*. 2004; 101:14192–14197. [PubMed: 15381778]
- Zhang Z, Ye G-Y, Cai J, Hu C. Comparative venom toxicity between *Pteromalus puparum* and *Nasonia vitripennis* (Hymenoptera: Pteromalidae) toward the hemocytes of their natural hosts, non-target insects and cultured insect cells. *Toxicon*. 2005; 46:337–349. [PubMed: 16026808]

Fig. 1.

Experimental design and comparisons conducted in present study. Each circle represents three replicates of five pooled hosts. (a) Comparisons to determine the effect of wounding by the ovipositor of *Nasonia*. (b) Comparisons within treatments to determine temporally changing gene expression. (c) Comparisons between treatments to determine the effects of *Nasonia* venom.

Fig. 2.

Multidimensional scaling analysis showing clustering of treatment and replicates. Squares are replicates from envenomated host transcriptions and circles from normal developing host transcriptomes. The number in each designates the hours after envenomation the sample was collected.

Fig. 3.

Comparing the number of differentially expressed genes in envenomated and normally developing hosts. (a) Number of significantly differentially expressed genes (adjusted $P < 0.05$) was determined by comparing 4-h hosts to each subsequent time point. (b) Overlap of differentially expressed genes among the temporally comparisons within envenomated and normally developing hosts as well as between the two treatments at each time point.

Fig. 4. Differential expression in fragments per kilobase per million (FPKM) of the *enhancer of split* complex between envenomated (solid line) and normally developing (dashed line) hosts at multiple time points.

Fig. 5.

Changes in pyruvate metabolism in envenomated hosts. Metabolites measured in Mrinalini *et al.* (2014) are highlighted within circles, and genes measured in current study are labelled by their gene abbreviations listed below. Arrows indicate the direction of significantly changing genes or metabolites in envenomated hosts. ACO, aconitase; AR, aldose reductase; CS, citrate synthase; FUM, fumarase; IDH, isocitrate dehydrogenase; LDH, lactate dehydrogenase; MDH, malate dehydrogenase; ME, malic enzyme; OGDH, 2-oxoglutarate dehydrogenase complex; PC, pyruvate carboxylase; PDC, pyruvate dehydrogenase complex; PEPC, phosphoenolpyruvate carboxylase; PK, pyruvate kinase; SCS, succinyl CoA ligase; SDH, sorbitol dehydrogenase; SQR, succinate dehydrogenase.

Table 1

Assembly and annotation information for the reference *Sarcophaga bullata* transcriptome at multiple filtering stages.

	All assembled transcripts	Longest transcript per loci	Longest transcript per loci filtered*
Number of contigs	113 689	14 697	8234
Average length	1427	1194	1974
BLASTX hit NCBI	—	7387 (50%)	6653 (81%)
BLAST hit to <i>Drosophila</i>	63181 (56%)	6802 (46%)	6475 (79%)
Hit to drosophila pupal expressed genes	—	—	5669 (69%)

* Longest transcript per loci filtered contains transcripts with >1 count per million reads