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Meta-Analysis of Immune Induced Gene Expression Changes in Diverse *Drosophila melanogaster* Innate Immune Responses

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Simple Summary: Organisms can be infected by a wide range of pathogens, including bacteria, viruses, and parasites. Following infection, the host mounts an immune response to attempt to eliminate the pathogen. These responses are often specific to the type of pathogen and mediated by the expression of specialized genes. We have characterized the expression changes induced in host *Drosophila* fruit flies following infection by multiple types of pathogens, and identified a small number of genes that show expression changes in each infection. This includes genes that are known to be involved in pathogen resistance, and others that have not been previously studied as immune response genes. These findings provide new insight into transcriptional changes that accompany *Drosophila* immunity. They may suggest possible roles for the differentially expressed genes in innate immune responses to diverse classes of pathogens, and serve to identify candidate genes for further empirical study of these processes.

Abstract: Organisms are commonly infected by a diverse array of pathogens and mount functionally distinct responses to each of these varied immune challenges. Host immune responses are characterized by the induction of gene expression, however, the extent to which expression changes are shared among responses to distinct pathogens is largely unknown. To examine this, we performed meta-analysis of gene expression data collected from *Drosophila melanogaster* following infection with a wide array of pathogens. We identified 62 genes that are significantly induced by infection. While many of these infection-induced genes encode known immune response factors, we also identified 21 genes that have not been previously associated with host immunity. Examination of the upstream flanking sequences of the infection-induced genes lead to the identification of two conserved enhancer sites. These sites correspond to conserved binding sites for GATA and nuclear factor κB (NFκB) family transcription factors and are associated with higher levels of transcript induction. We further identified 31 genes with predicted functions in metabolism and organismal development that are significantly downregulated following infection by diverse pathogens. Our study identifies conserved gene expression changes in *Drosophila melanogaster* following infection with varied pathogens, and transcription factor families that may regulate this immune induction.

Keywords: *Drosophila melanogaster*; innate immunity; gene expression; transcriptome analysis; pathogen infection

1. Introduction

Organisms encounter a broad range of pathogens in their natural environments and have evolved immune defenses that allow them to survive infection by diverse pathogen classes. Most metazoan hosts make use of a highly conserved suite of innate immune responses to defend against pathogen infection [1]. These responses are typified by a multi-step process, beginning with pathogen recognition and resulting in activation of the

appropriate immune mechanism [2]. This immune activation is accompanied by changes in gene expression, including both the induction of immune response transcripts and the downregulation of transcripts encoding proteins with non-immune functions [3,4]. The induced immune gene products then play a role in either eliminating the pathogen (pathogen resistance) or allowing the organism to survive despite infection (pathogen tolerance). Differential gene expression analysis has proven to be a valuable approach to uncover the genetic basis for a variety of traits [5–8], including the immune response to infection [9–11]. These studies have revealed the importance of conserved immune signaling pathways including Toll, Immune deficiency (IMD) and JAK-STAT across diverse organisms [12–19].

While hosts may encounter numerous distinct pathogenic species, the pathogens are often categorized into four general classes: bacterial pathogens, fungal pathogens, viruses, and parasites [20,21]. Innate immune responses can be highly specific to the category of pathogen encountered and may include both cellular and humoral mechanisms [2,22]. For instance, bacterial infection is often countered by the production of secreted antimicrobial peptides (AMPs) and immune cell mediated phagocytosis of the invading microbes [23,24]. Alternatively, antiviral immunity may include distinct features such as RNA interference or the recognition and cytolysis of infected host cells [25,26]. Highly conserved transcriptional signatures in response to distinct pathogen types have been uncovered, but less is known about common patterns of gene induction against multiple pathogens [27–30].

The genetic model organism *Drosophila melanogaster* is a commonly used and powerful system to understand conserved innate immune processes [31–33]. Like other animals, *Drosophila* mount specific responses to infection by distinct pathogen classes [20,22]. Numerous studies have investigated changes in gene expression in *Drosophila* hosts following infection by a wide range of pathogens including multiple species of bacterial and fungal pathogens, viruses, and parasitoid wasps (Table 1) [18,19,34–40]. These studies provide a unique opportunity for the comparative analysis of immune responses by a single host organism against diverse pathogens. A useful method to perform comparative analyses is the meta-analysis of gene expression studies. Such meta-analyses attempt to directly compare results from multiple studies while controlling for inter-study differences [41]. This approach is particularly useful as it allows for the reuse of existing datasets to address novel research questions, while providing a statistically rigorous framework [41]. Here, we use a common meta-analysis approach to perform a comparative analysis on multiple previously described *Drosophila* infection studies (Table 1) to identify genes whose expression are similarly altered across infection by distinct pathogen classes. Our meta-analysis approach allows us to take a broader view of infection induced transcriptional changes than would be otherwise possible, and extends the findings of the original studies.

Table 1. List of datasets used in the meta-analysis.

GEO Accession	Pathogen Type	Pathogen	Host Stage	Reference
-[1]	Bacteria	*Escherichia coli* + *Micrococcus luteus*	Adult	[34]
-[1]	Bacteria	*E. coli*, *M. luteus* + *Enterococcus faecalis*	Adult	[18]
GSE37708	Bacteria	*E. coli*	Adult	[35]
GSE5489	Bacteria	*E. coli*	Larva	[36]
-[1]	Fungus	*Beauvaria bassiana*	Adult	[34]
-[1]	Fungus	*Aspergillus fumigatus*	Adult	[18]
GSE2828	Virus	*Drosophila* C Virus	Adult	[37]
GSE42726	Virus	Sindbis virus (transgenic *Drosophila* model)	Adult	[38]
GSE31542	Virus	Flock House Virus	Adult	[39]
GSE31542	Virus	Sindbis virus	Adult	[39]
GSE25522	Parasite	*Ganaspis xanothopoda*	Larva	[40]
GSE8938	Parasite	*Leptopilina boulardi*	Larva	[19]

[1] Data available at http://www.fruitfly.org/expression/immunity/.

2. Materials and Methods

2.1. Drosophila Melanogaster Genome Data

Gene expression data analyzed in the current study are available through the Gene Expression Omnibus (https://www.ncbi.nlm.nih.gov/geo/ study accessions: GSE37708, GSE2828, GSE42726, GSE25522, GSE8938, GSE5489, GSE31542; accessed on 23 May 2014), and through the fruitfly.org Expression Database (http://www.fruitfly.org/expression/immunity/). Accession numbers and other metadata are listed in Table 1.

Gene expression data were then pre-processed before meta-analysis. First, gene identifiers were converted to the most recent FlyBase gene identification number (FBgn) using the FlyBase Upload and Validate IDs tool (version FB2021_01; https://flybase.org/convert/id) [42]. Second, the gene expression datasets were filtered to remove any genes that are not represented in all of the datasets. This step resulted in the identification of 10,818 common genes that were retained for subsequent analysis. Finally, gene expression fold change values were $\log_2$ transformed wherever necessary to be used as input for meta-analysis (next section). Data for the *D. melanogaster* genome (release 6.38) and individual gene reports were accessed through FlyBase (version FB2021_01; https://flybase.org/) [42].

2.2. Meta-Analysis of Gene Expression Studies

Meta-analysis of immune gene expression studies was performed in the R statistical computing environment [43], using the RankProd package [44,45]. The $\log_2$ fold change for each gene in each dataset was used as the input, and the rank and rank-product (RP) were calculated for each gene. Significance was determined using the estimated percentage of false prediction (pfp) with a threshold of 0.05. Genes with significantly altered expression are listed in Table S1 (62 upregulated genes) and Table S2 (31 downregulated genes). A control set of 62 genes with unchanged expression was selected from the RP result as listed in Table S4.

2.3. Chromosomal Distribution

The chromosomal location of each gene identified as significantly differentially expressed in the meta-analysis was retrieved from FlyBase (version FB2021_01). The proportion of identified genes found on each chromosomal arm was compared to the proportion of all genes for that arm using a 2-sample test for equality of proportions with continuity correction in R.

2.4. Gene Locus Uniformity and Clustering

To determine the uniformity of gene spacing across *D. melanogaster* chromosome arms, the up- and down-regulated gene lists were used as input for the Cluster Locator webserver (http://clusterlocator.bnd.edu.uy/ accessed on 24 April 2021) [46] using default parameters. Uniformity is tested using a two-sided Kolmogorov–Smirnov test. Clusters were identified using the Cluster Locator webserver with the default max-gap value of 5. Gene clustering is statistically tested by comparing clustering of the input lists with randomly selected lists.

2.5. Motif Finding

Putative transcription factor binding motifs were predicted using iMotifs [47,48]. The 250 bp of sequence immediately upstream of each gene of interest was downloaded from FlyBase, and these sequences were used as input to iMotifs. These predicted motifs were then mapped onto the input and unchanged control sequences using the FIMO tool [49]. To identify the likely transcription factor interacting with the discovered motifs, the motifs were compared with experimentally validated *D. melanogaster* transcription factor binding sites using the TomTom Motif Comparison Tool [50,51] with a significance threshold of $p < 0.05$. Position-weight-matrices for the identified transcription factor binding sites were accessed through the OnTheFly database [52]. OnTheFly accession numbers: GATAe: OTF0433.1; dl: OTF0107.2; Hr46/Hr3: OTF0227.1.

2.6. Statistics

All statistical tests were performed in R using the base stats package and graphs were produced using ggplot2 [53].

3. Results

3.1. Meta-Analysis of Genome-Wide Transcript Levels following Pathogen Infection

We performed a meta-analysis on 10,818 genes across 12 gene expression studies following infection by a variety of pathogens (listed in Table 1). To identify genes showing significant expression changes across these studies, we used the non-parametric rank products approach with an estimated percentage of false prediction (pfp) threshold of 0.05. In this method, the observed fold-change of each gene is ranked within each study and the rank-product of each gene is calculated as the geometric mean of the ranks of a given gene across all of the studies. Genes with rank-products that significantly differ from a uniform distribution are considered to be up- or down-regulated [45,54]. The use of ranks, rather than experimental values, makes this approach robust to differences between experimental platforms and allows for the comparison between multiple studies [44,45]. Using this approach, we identified 62 genes that were induced across these infection conditions (Table S1) with an average $\log_2$ fold change (logFC) of 1.17, and a logFC range of 0.62 to 2.27. We further identified 31 genes that were significantly downregulated across these infection conditions (Table S2). These downregulated genes have an average logFC of -0.75, with a logFC range of -0.26 to -1.40.

The identified genes were mapped onto their chromosomal locations (Figure 1) and found to be distributed throughout the autosomal chromosome arms, with few genes mapping to the X chromosome and none on chromosome 4. Given that only ~80 genes of ~18,000 total genes in the *D. melanogaster* genome are found chromosome 4 [55,56], the lack of immune regulated genes on chromosome 4 is unsurprising. On the other hand, the apparent lack of immune regulated genes on the X chromosome was unanticipated. We therefore used 2-sample proportion tests to assay the distribution of genes on each chromosome arm (Table 2). We find that induced genes are significantly enriched ($\chi^2 = 8.74$, $p = 0.0031$), and that downregulated genes are significantly under-represented ($\chi^2 = 4.56$, $p = 0.033$), on chromosome 2R. Additionally both induced and downregulated genes are under-represented on the X chromosome (induced: $\chi^2 = 3.88$, $p = 0.049$; downregulated: $\chi^2 = 11.13$, $p = 8.5 \times 10^{-4}$). This relative lack of genes was unexpected given the presence of numerous immune response genes on the X chromosome, although interestingly, the antimicrobial peptide class of immune effectors is also under-represented on the X chromosome [57].

Table 2. Distribution of analyzed genes across chromosome arms.

Sample	X	2L	2R	3L	3R	4	U	Total
Dataset	1793	1951	2129	2103	2734	65	43	10,818
Induced genes	4 [b]	11	22 [a]	8	17	0	0	62
Downregulated genes	0 [b]	6	5 [b]	9	11	0	0	31

The Dataset category contains the genes that were measured in all 12 datasets. [a] enriched relative to Dataset control, [b] under-represented relative to Dataset control; determined by $p < 0.05$ from 2-sample test for equality of proportions with continuity correction.

Next, we assayed the distribution of the identified genes within each chromosome arm for overall uniformity and the presence of gene clusters. Using the Kolmogorov–Smirnov test of uniformity, we find that the genes are evenly distributed along chromosomes (Table 3), but this analysis did identify the presence of 7 gene clusters. This represents a significant degree of clustering compared to background controls (induced $p = 1.76 \times 10^{-7}$; downregulated: $p = 0.003$). We identified 5 clusters of induced genes (annotated in Table S1) including the Bomanin family gene clusters (found on chromosomes 2R and 3R), clusters comprising the Diptericin (chromosome 2R) and Cecropin (chromosome 3R) antimicrobial

peptide families, and a cluster of two unstudied genes on chromosome 2L (*CG9928* and *CG16978*). We also identified 2 clusters of downregulated genes (annotated in Table S2) including a cluster of Trypsin genes (chromosome 2R) and a cluster of predicted S1A family serine protease genes (*CG18179* and *CG18180*; chromosome 3L).
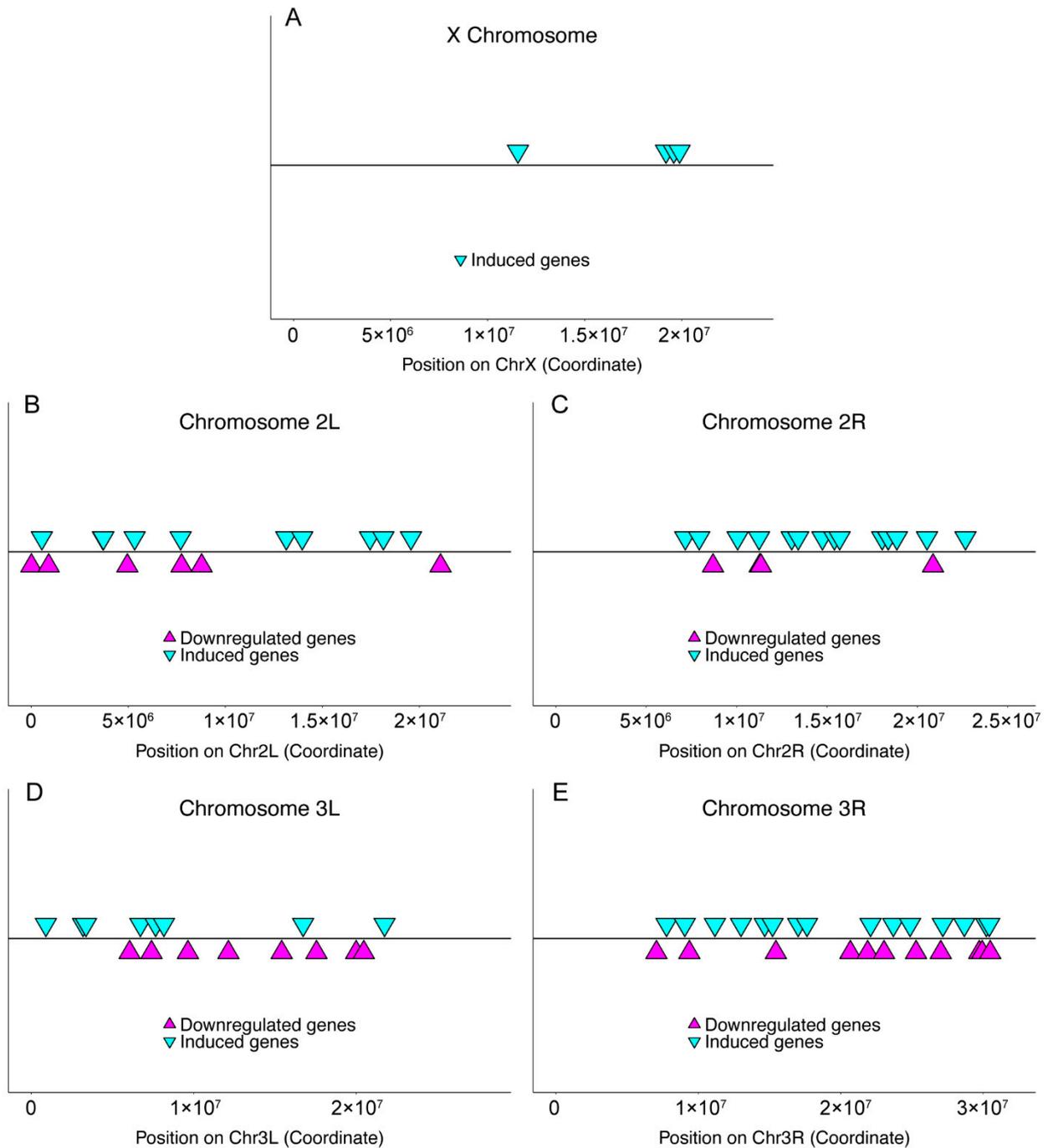
Figure 1. Chromosomal location of altered genes. Each identified gene has been mapped to its chromosomal location, indicated by its position on each chromosome arm of the *Drosophila melanogaster* genome (**A**–**E**). For each panel, the x axis represents the genomic position, inverted cyan triangles indicate the positions of induced genes and the magenta triangles indicate the positions of downregulated genes.

Table 3. Uniformity of altered genes within chromosome arms.

Chromosome Arm	*D* Up	*p* Value Up	*D* Down	*p* Value Down
X	0.62	0.05	-	-
2L	0.17	0.87	0.41	0.20
2R	0.24	0.15	0.51	0.10
3L	0.41	0.10	0.25	0.52
3R	0.17	0.66	0.31	0.19

The uniformities of induced and downregulated genes within each chromosome arm were independently assessed by Kolmogorov–Smirnov test. *D* is the calculated Kolmogorov–Smirnov distance, Up = induced (upregulated) genes, Down = downregulated genes.

### 3.2. Infection-Induced Genes in Host Immunity

Our meta-analysis identified 62 genes that are significantly upregulated following infection. Of these, 42 have been previously linked with host immunity (Table 4). This list includes genes that have been previously implicated in resistance to each of the pathogen categories. The list also includes genes with membership in the Toll, IMD, JAK-STAT and Jun N-terminal kinase (JNK) conserved immune signaling pathways. Accordingly, Gene Ontology (GO) term analysis revealed that the immune induced genes are enriched in multiple biological processes linked to responses to external stimuli including immune response (GO:0009655), response to biotic stimulus (GO:0009607), response to wounding (GO:0009611), and response to stress (GO:0006950) (Figure 2, Table S3).

Table 4. Infection induced genes with previous links to immune function or immune signaling pathways.

Gene Name	Function	Immune Pathway	References
AttA	Antimicrobial peptide	IMD	[58,59]
AttD	Antimicrobial peptide	IMD	[58]
Bbd	Production of AMP-like peptides	Toll	[60]
BomBc1	AMP-like	Toll	[61,62]
BomBc2	AMP-like	Toll	[61,62]
BomBc3	AMP-like	Toll	[61,62]
BomS1	AMP-like	Toll	[61–63]
BomS2	AMP-like	Toll	[61–63]
BomS3	AMP-like	Toll	[61–63]
BomS5	AMP-like	Toll	[61–63]
BomS6	AMP-like	Toll	[61–63]
BomT2	AMP-like	Toll	[61,62]
BomT3	AMP-like	Toll	[61,62]
CecB	Antimicrobial peptide	IMD	[64,65]
CecC	Antimicrobial peptide	IMD	[64,66]
Def	Antimicrobial peptide	IMD, Toll	[18,67]
DptA	Antimicrobial peptide	IMD	[64,68]
DptB	Antimicrobial peptide	IMD	[64,69]
Drs	Antimicrobial peptide	Toll	[70,71]
Ets21C	Transcription factor	IMD	[72]
BaraA	Antimicrobial peptide	Toll	[73]
Irc	Oxidant detoxification	-	[74]
lectin-24A	Carbohydrate binding	-	[75]
Listericin	Antimicrobial peptide	JAK-STAT	[76]
mat	-	JAK-STAT	[77,78]
Mtk	Antimicrobial peptide	IMD, Toll	[79,80]
nec	Serpin	Toll	[81]
NimB1	Pathogen recognition (predicted)	-	[82,83]
PGRP-SA	Pathogen recognition	Toll	[84,85]
PGRP-SB1	Antimicrobial effector	IMD	[86,87]
PGRP-SD	Pathogen recognition	Toll	[88]
Rel	Transcription factor	IMD	[89,90]

Table 4. *Cont.*

Gene Name	Function	Immune Pathway	References
Sid	DNA endonuclease	Toll	[18,91]
Sp7	S1A Serine Protease	Toll	[92,93]
SPE	S1A Serine Protease	Toll	[94,95]
Tep2	Thioester-containing Protein	Toll	[96]
TotM	-	JAK-STAT	[97,98]
CG13675	Chitin Binding	IMD	[99]
CG14957	Chitin Binding	JNK	[78]
CG3505	S1A Serine Protease	Toll/IMD	[100]
CG5909	S1A Serine Protease	Toll/IMD	[18]
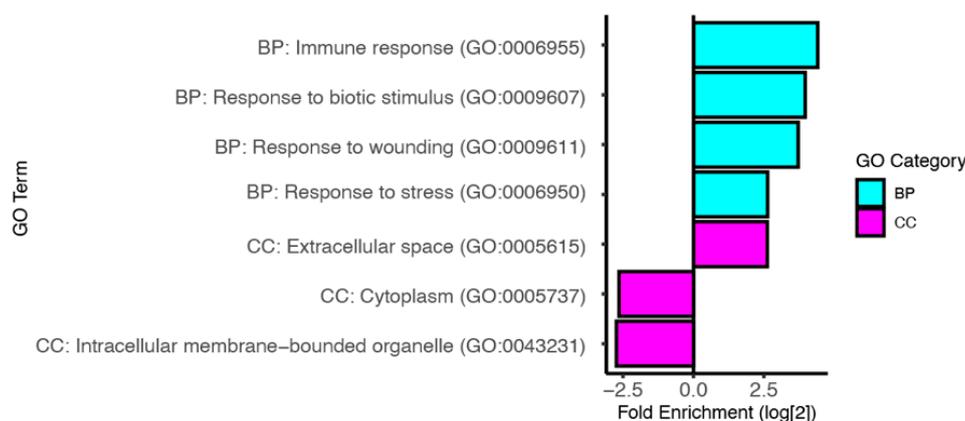
Figure 2. Gene ontology analysis of infection induced genes. The $\log_2$ fold enrichment for selected GO terms. The Biological Process (BP) category is shown in cyan, and the Cellular Component (CC) category is shown in magenta. See Table S3. for complete GO term analysis of induced genes.

Our meta-analysis results suggest that infection by a broad range of pathogens can lead to differential regulation of immune signaling pathways. We found that several genes implicated in pathogen recognition (*NimB1*, *lectin-24A* and *Tep2*), along with regulators of the Toll (*nec*, *PGRP-SA*, *SPE* and *Sp7*) and IMD (*PGRP-SD* and *Rel*) pathways, are induced following infection. We also identified a broad range of immune effector molecules including antimicrobial peptides (AMPs) and AMP-like immune induced genes. The *D. melanogaster* genome encodes numerous AMP families [101], and we identify members of nearly all of these families including the Attacins (AttA and AttD), Cecropins (CecB and CecC), Defensin, Diptericins (DptA and DptB), Drosomycin, IMPPP/Baramycin A, Listericin, and Metchnikowin (Table 4). This broad induction is particularly interesting given that the Toll and IMD signaling pathways and many of these AMP/AMP-like families have distinct pathogen targets [24].

Members of the *Bomanin* (*Bom*) AMP-like gene family have been shown to act down-stream of Toll pathway signaling in antimicrobial immunity [61,62]. We identified 10 of the 12 *Bom* family genes as induced in our meta-analysis (Table 4); of the other 2 *Bom* genes, *BomS4* is significantly induced following parasite infection, but not following infection by the other pathogens, and *BomT1* is not represented in our dataset. *Bom* genes are found in the genome in two clusters, and we identified genes from both clusters in our analysis. We additionally identified the *bombardier* (*bbd*) gene as induced in our analysis (Table 4). Like the *Bom* genes, *bbd* also acts in antimicrobial immunity downstream of Toll, and *bbd* mutants fail to produce the short-form class of *Bom* peptides (*BomS*) [60]. The finding that *bbd* is induced alongside *Bom* genes lends further support for a role for *Bom* family activity following infection.

### 3.3. Predicted Functions of Infection Induced Genes

The proteins encoded by the infection induced genes have a wide array of predicted molecular functions. This includes both immune associated functions like antimicrobial activity and peptidoglycan recognition, and a variety of other functions such as ion transport (*MFS12*), deoxyribonuclease activity (*Sid*), and acyl transferase activity (*CG14219*). Notably, we identified 5 members of the S1A protease family (*SPE*, *Sp7*, *CG3505*, *CG18563* and *CG5909*). The S1A family is comprised of more than 200 genes and includes both active proteases and catalytically inactive protease homologs [102]. S1A family members have been previously linked to immune responses against a variety of pathogens [84,94,103–105]. Due to the wide array of encoded protein activities, our GO term analysis did not identify any significant enrichment for molecular function. However, we did identify an enrichment of genes encoding proteins that are secreted into the extracellular space (GO:0005615) and an under-representation of genes encoding cytosolic (GO:0005737) and intracellular membrane-bounded organelle localized proteins (GO:0043231) (Figure 2, Table S3).

3.4. Motif Finding Analysis of Infection Induced Genes

The timing and levels of mRNA transcription are tightly regulated by the activity of a wide array of transcription factors. Multiple transcription factor families, including the nuclear factor κB (NFκB), nuclear factor of activated T cells (NFAT), signal transducer and activator of transcription (STAT) and erythroblast transformation specific (ETS) factors have been linked to transcription induction following infection [106–109]. We predicted that the induced genes uncovered in our meta-analysis are co-transcriptionally regulated, and share a common set of transcription factors. To test this prediction, we analyzed the 250 bp of genomic sequence upstream of the annotated transcription start site of each of the induced genes using the iMotifs de novo motif finder [47]. We reasoned that these sequences likely included the promoter and proximal enhancers for each gene, and our approach would allow us to test whether conserved binding motifs for any of these transcription factor families are found in the immune induced genes, and uncover any motif that was found in the upstream region of the majority of the induced genes.

Our analysis led to the identification of 3 consensus motifs (Figure 3A–C). These consensus sequences were searched against the complete database of known *D. melanogaster* transcription factor binding sites using the Tomtom web server [51]. We found that our Motif 1 showed significant similarity to the binding site for the GATA factor *GATAe* ($p = 6.0 \times 10^{-6}$; Figure 3A), and that our Motif 2 showed significant similarity to the binding site for the NFκB factor *dl* ($p = 5.6 \times 10^{-4}$; Figure 3B). It can be challenging to correctly identify the binding site for a specific member for either of these transcription factor families [110,111], and so we will refer to the sites by their general classifications as GATA and NFκB sites for Motifs 1 and 2, respectively. We did not find motifs that show similarity to the NFAT, STAT or ETS transcription factor families, and Motif 3 did not show significant similarity to any known *D. melanogaster* transcription factor binding site. However, its core sequence matches the TATA box characteristic of many eukaryotic core promoters (Figure 3C) [112,113]. This finding supports our use of upstream genomic sequences to capture gene promoter regions.

Since both GATA and NFκB factors have been previously linked to infection induced transcription in *Drosophila* [89,114–116], we next tested whether these sites are more common in the upstream regions of our induced genes than of a control list of unchanged genes from the meta-analysis (Table S4). We find that our identified induced genes are significantly more likely that the background control to contain either a Motif 1/GATA or Motif 2/NFκB site (Fisher's Exact Test odds ratio 7.5, $p = 1.6 \times 10^{-6}$). More specifically, both Motif 1 and Motif 2 are enriched in the upstream regions of our induced genes (Motif 1: odds ratio 4.7, $p = 1.0 \times 10^{-4}$; Motif 2: odds ratio 5.2, $p = 2.5 \times 10^{-5}$; Table 5), and the induced genes are also significantly more likely to contain both motifs (odds ratio 16.2, $p = 5.3 \times 10^{-6}$; Table 5).
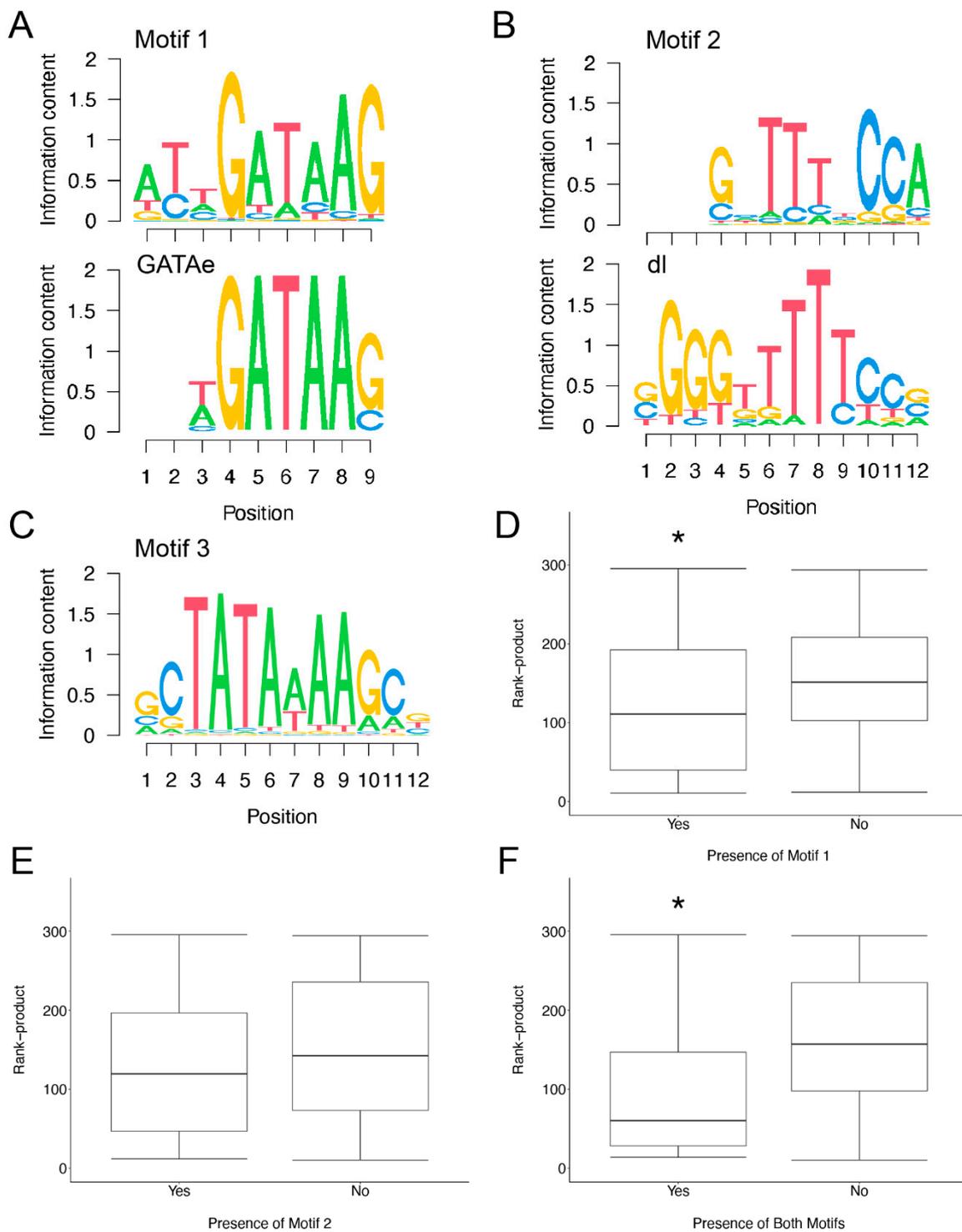
Figure 3. Motifs associated with infection induced genes. De novo motif finding identified 3 motifs (Motifs 1–3) that are enriched in the upstream sequences of the induced genes. The consensus motifs are represented as sequence logos (**A**–**C**, top). Motif matching identifies Motif 1 as being significantly similar to the GATAe binding site (**A**). Motif 2 shows significant similarity to the dl binding site (**B**). (**D**–**F**) Box-whisker plots showing the distribution of rank-products for induced genes with and without the indicated motifs. A lower rank-product is indicative of higher expression levels. (**D**) Induced genes with Motif 1 in the upstream region have significantly lower rank-products. (**E**) The presence of Motif 2 does not impact the rank-product distribution. (**F**) Induced genes with both motifs have significantly lower rank-products. Asterisk (*) indicates $p < 0.05$ relative to induced genes without the indicated motif.

Table 5. Enrichment of predicted transcription factor binding sites in the induced genes compared to unchanged control genes.

Gene Set	Motif 1	Motif 2	Both	Neither	Total
Induced	36 *	39 *	22 *	9 *	62
Unchanged	14	15	2	35	62

* $p > 0.05$ compared to unchanged control by Fisher's exact test.

While this enrichment is suggestive that GATA and NFκB factors have an impact on immune induced expression, we wanted to test this hypothesis more explicitly. We used the Wilcoxon rank sum exact test to test the effect of each site on the rank-product of the induced genes. We would predict that if the presence of Motif 1/GATA or Motif 2/NFκB (or both) sites has a positive effect on expression, then transcripts with these sites would have significantly lower rank-products (indicative of higher expression). We find that genes with Motif 1 have significantly lower rank-products that those without (W = 595, $p = 0.035$; Figure 3D), while the presence of Motif 2 alone has little to no impact on rank-product (W = 510, $p = 0.189$; Figure 3E). In contrast, transcripts with both motifs have significantly lower rank-products (W = 616, $p = 4.53 \times 10^{-3}$; Figure 3F), with the strength of this effect hinting at a possible synergistic effect of GATA and NFκB factors.
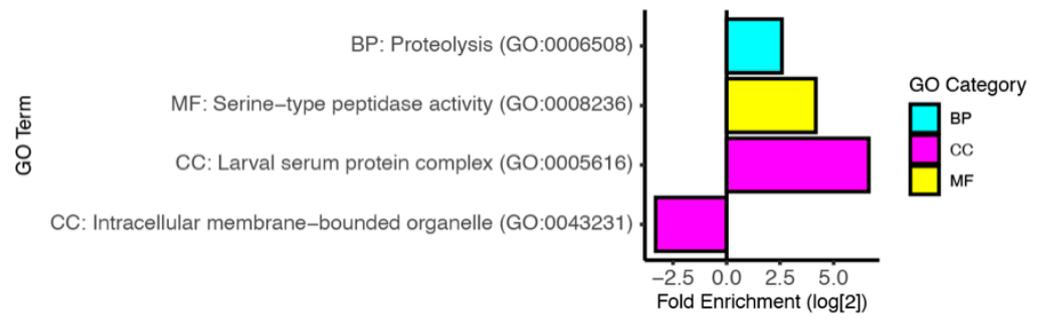
3.5. Analysis of Downregulated Transcripts

Our meta-analysis identified 31 genes that are significantly downregulated following infection with the various pathogens (Table S2). Most of these genes may be predicted to be influenced by life history tradeoffs that occur following infection. Mounting an immune response is energetically costly, and following infection organismal metabolism is altered [117–119]. We find a wide range of genes linked to metabolism are downregulated following infection including genes linked to amino acid metabolism (*Lsp1β*, *Lsp2*, *Srr*), lipid metabolism (*CG17192*, *mag*), and carbohydrate metabolism (*LManVI*, *Sodh-1*). The shifting of resources towards immunity is often at the expense of organismal development or fertility [120–122], and among our downregulated genes, we find genes associated with these processes including *fln*, *Act88F*, *CG33259*, *Cpr92F*, and *TpnC47D*.

In many organisms, pathogen infection leads to coordinated changes in host physiology and behaviour, known as sickness behaviour [123]. These changes include decreased host appetite and feeding following infection in a wide range of host species, including *Drosophila* [123–127]. Accordingly, we find that genes involved in feeding behaviour (*fit*) and nutritional stress (*CG18179*, *CG18180*), along with putative digestive enzymes in the Trypsin and Jonah protease families are all downregulated following pathogen infection [128–130]. Interestingly, despite the widespread prevalence of infection induced anorexia, this mechanism is not uniformly protective, and instead can lower host resistance to certain pathogens [127,131,132].

Our GO term analysis (Figure 4, Table S5) of these downregulated genes reveals an enrichment in genes involved in proteolysis (GO:0006508), and specifically in serine-type peptidase activity (GO:0008236). The enrichment in genes found in the larval serum protein complex (GO:0005616) likely reflects the observed metabolic change. Like with the induced genes, we also find an under-representation of genes encoding proteins that localize to intracellular membrane-bounded organelles (GO:0043231).

We used motif finding software to scan the 250 bp regions upstream of the transcription start sites of the downregulated genes, and identified a putative transcription factor binding site (Motif D1, Figure 5A). This motif is homologous to the identified Hr3 binding site ($p = 0.005$; Figure 5A). However, we find that Motif D1 is not enriched among downregulated genes in comparison with our unchanged control gene set (odds ratio 1.14, $p = 0.826$), and that the presence of Motif D1 does not have a significant effect on the rank-product among downregulated genes (W = 97, $p = 0.811$; Figure 5B), suggesting that this site is likely not mediating the downregulation observed following infection.

Figure 4. Gene ontology analysis of downregulated genes. The $\log_2$ fold enrichment for selected GO terms. The Biological Process (BP) category is shown in cyan, the Molecular Function (MF) category is shown in yellow, and the Cellular Component (CC) category is shown in magenta. See Table S5 for complete GO term analysis of downregulated genes.
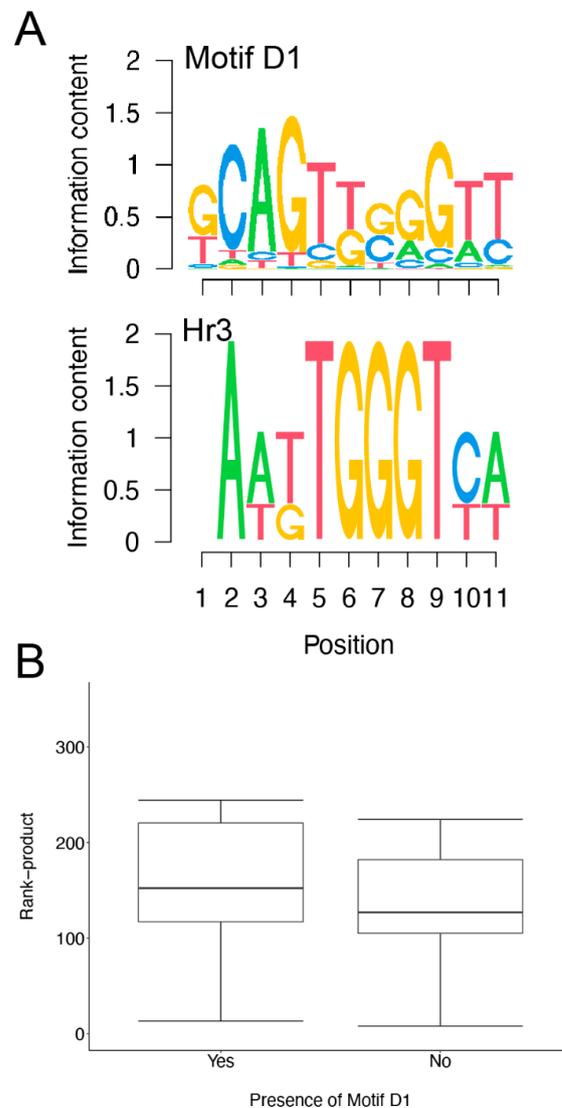
Figure 5. Motif associated with downregulated genes. De novo motif finding identified 1 motif (Motif D1) that is enriched in the upstream sequences of the induced genes. The consensus motif is represented as a sequence logo (**A**, top). Motif matching identifies Motif D1 as being significantly similar to the Hr3 binding site (**A**). (**B**) Box-whisker plot showing the distribution of rank-products for the downregulated genes with and without Motif D1. The presence of Motif D1 does not impact the rank-product distribution.

4. Discussion

Our findings have uncovered a subset of *Drosophila melanogaster* genes whose expression is altered following infection by a range of pathogens. These genes are likely mediating known responses to infection, for instance we find that known immune response genes predominate among the induced genes on our list. Many of the downregulated genes we identified are linked to metabolism, feeding, development, and reproduction; processes which are all altered following infection. Additionally, our analysis has uncovered a putative transcriptional mechanism that regulates gene expression following infection with diverse pathogens.

In the absence of in vivo experimental data, we are unable to draw conclusions about the roles of the genes we've identified, however our bioinformatic and meta-analyses do allow us to generate interesting hypotheses for future testing. We believe that our lists of induced and downregulated genes can provide insight in host immunity, particularly due to the presence of genes with experimentally defined functions that align with observed immune response mechanisms as highlighted below; however, these roles need to be empirically tested in future studies. Hopefully our analyses have provided an interesting list of candidate genes whose study can begin to unravel important immune mechanisms.

Intriguingly, we find that many specific immune effector encoding genes are induced following infection by diverse pathogens. For instance, antimicrobial and AMP-like peptides are not known to play a role in the antiparasite immune response, and yet are induced following parasitoid wasp infection (Table 4) [19,133,134]. A possible model to explain this observation is that the *Drosophila* genome encodes a conserved set of immune effector genes that act against all types of pathogen infection. This model is unlikely given the long history of findings suggesting that specific immune effectors are used to target distinct pathogens [24,135]. Additionally, multiple studies have demonstrated that selection for flies with resistance to a particular pathogen does not translate to cross-resistance to additional pathogens [136,137]. Specifically, flies selected for resistance to the parasitoid *Asobara tabida* do not show increased resistance to bacterial or fungal pathogens [136], and fly lines selected for resistance to the bacterial pathogen *Bacillus cereus* do not display cross-resistance to Sigma virus [137].

Instead, our findings may suggest a second model in which sensing pathogen infection leads to the induction of genes that play a role in surveillance and resistance to possible coinfecting pathogens. Coinfections are commonly observed in natural populations of various species [138–140]. It has been demonstrated that coinfection can lead to decreased host resistance in nature and laboratory experiments [140–142], and negatively impact host health [139,143]. These previous findings suggest that avoidance of coinfection would increase host fitness, and support a model in which infection by any pathogen may provoke a generalized prophylactic response against coinfection alongside the specific response to the primary pathogen. Indeed, we have identified a large number of induced genes that encode immune recognition proteins and regulators of the Toll, IMD, and JAK-STAT immune signaling pathways, along with immune effectors that target distinct classes of pathogens. These pathways are among the main immune response pathways in *Drosophila*, and in combination with the breadth of induced immune effectors, our findings suggest that infected flies are primed to respond to the possibility of coinfection.

This model is also supported by previous findings. For instance, AMP expression is seen at early time points following parasitoid infection [19,133], but little to no expression of antimicrobial immune effectors is observed at late time points following parasitoid infection [134]. These findings may make sense in the light of the coinfection prevention model. In nature, *Drosophila* larvae are found in the microbe-rich environment of decaying fruit [144]. Parasitoid infection of *Drosophila* larvae results in the wasp ovipositor puncturing a hole in the larval cuticle; this wound will be healed, however the healing of epidermal wounds can take several hours [145,146]. Immediately following parasitoid infection, and before healing is complete, the wound can therefore provide a readily available infection route for environmental microorganisms. The expression of antimicrobial

factors and surveillance for any surviving microbes may therefore play an important role in pre-empting this possible route of coinfection.

Additional support for this model is provided by an in-depth time course study of the transcriptional response to IMD pathway activation [147]. In this study, stimulation of the IMD pathway resulted in the expression of Toll pathway regulated genes, including *Bom* family genes, and stress response genes, including *TotM*, all of which we also identified in our meta-analysis. Interestingly, the high resolution time course provided by this study illustrates that the Toll and stress response genes were induced as part of an early and transient response to IMD pathway stimulation, in contrast to the sustained transcription of known IMD responsive genes [147]. This pattern would fit with our expectations under a coinfection prevention model, in which immune stimulation simultaneously triggers a specific response against the identified pathogen (IMD pathway genes) and leads to the production of a temporary prophylactic state (Toll and stress response genes) to guard against possible coinfection.

Using de novo motif finding analysis, we identified putative binding sites for NFκB and GATA family transcription factors. The degree of gene induction in our meta-analysis correlates with the presence of these factors, and suggests a possible synergistic relationship between them. NFκB and GATA factor activity have been linked to immune responses, and have been previously demonstrated to work in concert to promote immune gene expression [148], supporting our idea that these factors may be underlying the response to infection by diverse pathogens. The *Drosophila* genome encodes three NFκB family genes (*Dif*, *dl*, *Rel*), all of which have been previously linked to immunity, and 5 GATA factors (*pnr*, *srp*, *grn*, *dGATAd*, *dGATAe*) of which *srp* and *dGATAe* have been previously linked to immune responses [89,114–116,149]. The difficulty in distinguishing between paralog-specific binding site motifs within these families leaves us unable to speculate whether the response is driven by a particular family member, or whether multiple members may play a role. Cross-regulation of gene expression has been observed between the NFκB-dependent Toll (*Dif* and *dl* dependent) and IMD (*Rel* dependent) pathways [150], perhaps suggesting some redundancy between NFκB factors. In order to build a model of transcriptional regulation the role of individual factors must still be tested experimentally, and the results may help in understanding the immune response to infection.

While downregulated genes are often overlooked in studies of gene expression, they may still provide insight into the process being studied. Our meta-analysis identified a small number of transcripts that are downregulated following infection by diverse pathogens. The functions of these genes suggest that they may be playing a role in the switch to an altered metabolic state following infection. Infected flies display altered feeding behaviour, and prioritize using energy resources for immunity ahead of development or reproduction [120,121,127,151]. Accordingly, we find that genes previously linked with these processes are downregulated in infected flies. The further study of these genes may shed light on the largely unknown mechanisms underlying the life history tradeoffs induced by pathogen infection.

5. Conclusions

Our meta-analysis has identified 93 genes whose transcript levels are significantly altered following infection by diverse pathogens. Analysis of the experimentally determined and predicted functions of the proteins encoded by these genes suggests that they may play a role in immune function, immune metabolism and infection induced life history tradeoffs. Follow up studies on the roles of these genes following infection will be necessary to verify their importance and will likely improve our understanding of conserved immune functions.

enriched Gene Ontology terms among genes identified as induced by the RankProduct analysis; Table S4: Genes identified as unchanged from the RankProduct analysis and used as a representative background set for motif analysis; Table S5: All significantly enriched Gene Ontology terms among genes identified as downregulated by the RankProduct analysis.

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References

1. Danilova, N. The evolution of immune mechanisms. *J. Exp. Zoöl. Part B Mol. Dev. Evol.* **2006**, *306*, 496–520. [CrossRef] [PubMed]
2. Chaplin, D.D. Overview of the immune response. *J. Allergy Clin. Immunol.* **2010**, *125*, S3–S23. [CrossRef] [PubMed]
3. Fairfax, B.P.; Knight, J.C. Genetics of gene expression in immunity to infection. *Curr. Opin. Immunol.* **2014**, *30*, 63–71. [CrossRef] [PubMed]
4. Zhang, Q.; Cao, X. Epigenetic regulation of the innate immune response to infection. *Nat. Rev. Immunol.* **2019**, *19*, 417–432. [CrossRef]
5. Groitl, B.; Dahl, J.-U.; Schroeder, J.W.; Jakob, U. Pseudomonas aeruginosa defense systems against microbicidal oxidants. *Mol. Microbiol.* **2017**, *106*, 335–350. [CrossRef]
6. Kar, S.; Mai, H.-J.; Khalouf, H.; Ben Abdallah, H.; Flachbart, S.; Fink-Straube, C.; Bräutigam, A.; Xiong, G.; Shang, L.; Panda, S.K.; et al. Comparative Transcriptomics of Lowland Rice Varieties Uncovers Novel Candidate Genes for Adaptive Iron Excess Tolerance. *Plant Cell Physiol.* **2021**, *62*, 624–640. [CrossRef]
7. Nica, A.C.; Dermitzakis, E.T. Using gene expression to investigate the genetic basis of complex disorders. *Hum. Mol. Genet.* **2008**, *17*, R129–R134. [CrossRef]
8. Petruccelli, E.; Brown, T.; Waterman, A.; Ledru, N.; Kaun, K.R. Alcohol Causes Lasting Differential Transcription in Drosophila Mushroom Body Neurons. *Genetics* **2020**, *215*, 103–116. [CrossRef]
9. Scheid, A.D.; Van Keulen, V.P.; Felts, S.J.; Neier, S.C.; Middha, S.; Nair, A.A.; Techentin, R.W.; Gilbert, B.K.; Jen, J.; Neuhauser, C.; et al. Gene Expression Signatures Characterized by Longitudinal Stability and Interindividual Variability Delineate Baseline Phenotypic Groups with Distinct Responses to Immune Stimulation. *J. Immunol.* **2018**, *200*, 1917–1928. [CrossRef]
10. Mola, S.; Foisy, S.; Boucher, G.; Major, F.; Beauchamp, C.; Karaky, M.; Goyette, P.; Lesage, S.; Rioux, J.D. A transcriptome-based approach to identify functional modules within and across primary human immune cells. *PLoS ONE* **2020**, *15*, e0233543. [CrossRef]
11. Ding, P.; Ngou, B.P.M.; Furzer, O.J.; Sakai, T.; Shrestha, R.K.; MacLean, D.; Jones, J.D.G. High-resolution expression profiling of selected gene sets during plant immune activation. *Plant Biotechnol. J.* **2020**, *18*, 1610–1619. [CrossRef] [PubMed]
12. Edgerton, E.B.; McCrea, A.R.; Berry, C.T.; Kwok, J.Y.; Thompson, L.K.; Watson, B.; Fuller, E.M.; Nolan, T.J.; Lok, J.B.; Povelones, M. Activation of mosquito immunity blocks the development of transmission-stage filarial nematodes. *Proc. Natl. Acad. Sci. USA* **2020**, *117*, 3711–3717. [CrossRef] [PubMed]
13. Brutscher, L.M.; Daughenbaugh, K.F.; Flenniken, M.L. Virus and dsRNA-triggered transcriptional responses reveal key components of honey bee antiviral defense. *Sci. Rep.* **2017**, *7*, 1–15. [CrossRef]
14. Yang, X.; Fors, L.; Slotte, T.; Theopold, U.; Binzer-Panchal, M.; Wheat, C.W.; Hambäck, P.A. Differential Expression of Immune Genes between Two Closely Related Beetle Species with Different Immunocompetence following Attack by Asecodes parviclava. *Genome Biol. Evol.* **2020**, *12*, 522–534. [CrossRef] [PubMed]

15.　Roesel, C.L.; Vollmer, S.V. Differential gene expression analysis of symbiotic and aposymbiotic Exaiptasia anemones under immune challenge with Vibrio coralliilyticus. *Ecol. Evol.* **2019**, *9*, 8279–8293. [CrossRef]

16.　Byadgi, O.; Chen, Y.-C.; Maekawa, S.; Wang, P.-C.; Chen, S.-C. Immune-Related Functional Differential Gene Expression in Koi Carp (*Cyprinus carpio*) after Challenge with *Aeromonas sobria*. *Int. J. Mol. Sci.* **2018**, *19*, 2107. [CrossRef] [PubMed]

17.　Robledo, D.; Taggart, J.B.; Ireland, J.H.; McAndrew, B.J.; Starkey, W.G.; Haley, C.S.; Hamilton, A.; Guy, D.R.; Mota-Velasco, J.C.; Gheyas, A.A.; et al. Gene expression comparison of resistant and susceptible Atlantic salmon fry challenged with Infectious Pancreatic *Necrosis virus* reveals a marked contrast in immune response. *BMC Genom.* **2016**, *17*, 279. [CrossRef]

18.　De Gregorio, E.; Spellman, P.T.; Tzou, P.; Rubin, G.; Lemaitre, B. The Toll and Imd pathways are the major regulators of the immune response in Drosophila. *EMBO J.* **2002**, *21*, 2568–2579. [CrossRef]

19.　Schlenke, T.A.; Morales, J.; Govind, S.; Clark, A. Contrasting Infection Strategies in Generalist and Specialist Wasp Parasitoids of *Drosophila melanogaster*. *PLoS Pathog.* **2007**, *3*, e158. [CrossRef]

20.　Brennan, C.A.; Anderson, K.V. *Drosophila*: The Genetics of Innate Immune Recognition and Response. *Annu. Rev. Immunol.* **2004**, *22*, 457–483. [CrossRef]

21.　Crum-Cianflone, N.F. Bacterial, Fungal, Parasitic, and Viral Myositis. *Clin. Microbiol. Rev.* **2008**, *21*, 473–494. [CrossRef] [PubMed]

22.　Lemaitre, B.; Hoffmann, J. The Host Defense of *Drosophila melanogaster*. *Annu. Rev. Immunol.* **2007**, *25*, 697–743. [CrossRef] [PubMed]

23.　Gordon, S.; Plüddemann, A.; Estrada, F.M. Macrophage heterogeneity in tissues: Phenotypic diversity and functions. *Immunol. Rev.* **2014**, *262*, 36–55. [CrossRef] [PubMed]

24.　Lemaitre, B.; Reichhart, J.-M.; Hoffmann, J.A. *Drosophila* host defense: Differential induction of antimicrobial peptide genes after infection by various classes of microorganisms. *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 14614–14619. [CrossRef] [PubMed]

25.　Koonin, E.V. Evolution of RNA- and DNA-guided antivirus defense systems in prokaryotes and eukaryotes: Common ancestry vs. convergence. *Biol. Direct* **2017**, *12*, 5. [CrossRef]

26.　Weber, F. Antiviral Innate Immunity: Introduction. *Encycl. Virol.* **2020**, 577–583. [CrossRef]

27.　Beutler, B.; Jiang, Z.; Georgel, P.; Crozat, K.; Croker, B.; Rutschmann, S.; Du, X.; Hoebe, K. GENETIC ANALYSIS OF HOST RESISTANCE: Toll-Like Receptor Signaling and Immunity at Large. *Annu. Rev. Immunol.* **2006**, *24*, 353–389. [CrossRef]

28.　Lemaitre, B.; Nicolas, E.; Michaut, L.; Reichhart, J.-M.; Hoffmann, J.A. The Dorsoventral Regulatory Gene Cassette spätzle/Toll/cactus Controls the Potent Antifungal Response in Drosophila Adults. *Cell* **1996**, *86*, 973–983. [CrossRef]

29.　Medzhitov, R. Toll-like receptors and innate immunity. *Nat. Rev. Immunol.* **2001**, *1*, 135–145. [CrossRef]

30.　O'Shea, J.J.; Plenge, R. JAK and STAT Signaling Molecules in Immunoregulation and Immune-Mediated Disease. *Immunity* **2012**, *36*, 542–550. [CrossRef]

31.　Hoffmann, J.A.; Kafatos, F.C.; Janeway, C.A.; Ezekowitz, R.A.B. Phylogenetic Perspectives in Innate Immunity. *Science* **1999**, *284*, 1313–1318. [CrossRef] [PubMed]

32.　Howell, L.; Sampson, C.J.; Xavier, M.J.; Bolukbasi, E.; Heck, M.M.S.; Williams, M.J. A directed miniscreen for genes involved in the Drosophila anti-parasitoid immune response. *Immunogenetics* **2011**, *64*, 155–161. [CrossRef] [PubMed]

33.　Stroschein-Stevenson, S.L.; Foley, E.; O'Farrell, P.H.; Johnson, A.D. Identification of Drosophila Gene Products Required for Phagocytosis of Candida albicans. *PLoS Biol.* **2005**, *4*, e4. [CrossRef] [PubMed]

34.　De Gregorio, E.; Spellman, P.T.; Rubin, G.M.; Lemaitre, B. Genome-wide analysis of the *Drosophila* immune response by using oligonucleotide microarrays. *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 12590–12595. [CrossRef] [PubMed]

35.　Felix, T.M.; Hughes, K.A.; Stone, E.A.; Drnevich, J.M.; Leips, J. Age-Specific Variation in Immune Response in *Drosophila melanogaster* Has a Genetic Basis. *Genetics* **2012**, *191*, 989–1002. [CrossRef]

36.　Pal, S.; Wu, J.; Wu, L.P. Microarray analyses reveal distinct roles for Rel proteins in the Drosophila immune response. *Dev. Comp. Immunol.* **2008**, *32*, 50–60. [CrossRef]

37.　Dostert, C.; Jouanguy, E.; Irving, P.; Troxler, L.; Galiana-Arnoux, D.; Hetru, C.; Hoffmann, J.A.; Imler, J.-L. The Jak-STAT signaling pathway is required but not sufficient for the antiviral response of drosophila. *Nat. Immunol.* **2005**, *6*, 946–953. [CrossRef]

38.　Huang, Z.; Kingsolver, M.B.; Avadhanula, V.; Hardy, R.W. An Antiviral Role for Antimicrobial Peptides during the Arthropod Response to Alphavirus Replication. *J. Virol.* **2013**, *87*, 4272–4280. [CrossRef]

39.　Kemp, C.; Mueller, S.; Goto, A.; Barbier, V.; Paro, S.; Bonnay, F.; Dostert, C.; Troxler, L.; Hetru, C.; Meignin, C.; et al. Broad RNA Interference–Mediated Antiviral Immunity and Virus-Specific Inducible Responses in *Drosophila*. *J. Immunol.* **2012**, *190*, 650–658. [CrossRef]

40.　Lee, M.J.; Mondal, A.; Small, C.; Paddibhatla, I.; Kawaguchi, A.; Govind, S. A database for the analysis of immunity genes in Drosophila. *Fly* **2011**, *5*, 155–161. [CrossRef]

41.　Toro-Domínguez, D.; García, J.A.V.; Martorell-Marugán, J.; Román-Montoya, Y.; Alarcón-Riquelme, M.E.; Carmona-Sáez, P. A survey of gene expression meta-analysis: Methods and applications. *Brief. Bioinform.* **2020**, *22*, 1694–1705. [CrossRef] [PubMed]

42.　Larkin, A.; Marygold, S.J.; Antonazzo, G.; Attrill, H.; dos Santos, G.; Garapati, P.V.; Goodman, J.L.; Gramates, L.S.; Millburn, G.; Strelets, V.B.; et al. FlyBase: Updates to the *Drosophila melanogaster* knowledge base. *Nucleic Acids Res.* **2020**, *49*, D899–D907. [CrossRef] [PubMed]

43.　R Core Team. *R: A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria, 2021.

44. Del Carratore, F.; Jankevics, A.; Eisinga, R.; Heskes, T.; Hong, F.; Breitling, R. RankProd 2.0: A refactored bioconductor package for detecting differentially expressed features in molecular profiling datasets. *Bioinformatics* **2017**, *33*, 2774–2775. [CrossRef] [PubMed]

45. Hong, F.; Breitling, R.; McEntee, C.W.; Wittner, B.S.; Nemhauser, J.L.; Chory, J. RankProd: A bioconductor package for detecting differentially expressed genes in meta-analysis. *Bioinformatics* **2006**, *22*, 2825–2827. [CrossRef]

46. Obregón, F.P.; Soto, P.; Lavín, J.L.; Cortazar, A.R.; Barrio, R.; Aransay, A.M.; Cantera, R. Cluster Locator, online analysis and visualization of gene clustering. *Bioinformatics* **2018**, *34*, 3377–3379. [CrossRef]

47. Piipari, M.; Down, T.A.; Saini, H.; Enright, A.; Hubbard, T.J. iMotifs: An integrated sequence motif visualization and analysis environment. *Bioinformatics* **2010**, *26*, 843–844. [CrossRef]

48. Ryan, S.M.; Wildman, K.; Oceguera-Perez, B.; Barbee, S.; Mortimer, N.T.; Vrailas-Mortimer, A.D. Evolutionarily conserved transcription factors drive the oxidative stress response in Drosophila. *J. Exp. Biol.* **2020**, *223*, jeb221622. [CrossRef]

49. Grant, C.E.; Bailey, T.L.; Noble, W.S. FIMO: Scanning for occurrences of a given motif. *Bioinformatics* **2011**, *27*, 1017–1018. [CrossRef]

50. Bailey, T.L.; Johnson, J.; Grant, C.E.; Noble, W.S. The MEME Suite. *Nucleic Acids Res.* **2015**, *43*, W39–W49. [CrossRef]

51. Gupta, S.; Stamatoyannopoulos, J.A.; Bailey, T.L.; Noble, W.S. Quantifying similarity between motifs. *Genome Biol.* **2007**, *8*, R24. [CrossRef]

52. Shazman, S.; Lee, H.; Socol, Y.; Mann, R.S.; Honig, B. OnTheFly: A database of *Drosophila melanogaster* transcription factors and their binding sites. *Nucleic Acids Res.* **2013**, *42*, D167–D171. [CrossRef] [PubMed]

53. Wickham, H. *Ggplot2: Elegant Graphics for Data Analysis*; Springer: New York, NY, USA, 2009; ISBN 978-0-387-98141-3.

54. Breitling, R.; Armengaud, P.; Amtmann, A.; Herzyk, P. Rank products: A simple, yet powerful, new method to detect differentially regulated genes in replicated microarray experiments. *FEBS Lett.* **2004**, *573*, 83–92. [CrossRef] [PubMed]

55. Riddle, N.C.; Elgin, S.C.R. The *Drosophila* Dot Chromosome: Where Genes Flourish Amidst Repeats. *Genetics* **2018**, *210*, 757–772. [CrossRef]

56. Kaufman, T.C. A Short History and Description of *Drosophila melanogaster* Classical Genetics: Chromosome Aberrations, Forward Genetic Screens, and the Nature of Mutations. *Genetics* **2017**, *206*, 665–689. [CrossRef] [PubMed]

57. Hill-Burns, E.M.; Clark, A.G. X-Linked Variation in Immune Response in *Drosophila melanogaster*. *Genetics* **2009**, *183*, 1477–1491. [CrossRef]

58. Hedengren, M.; Borge, K.; Hultmark, D. Expression and Evolution of the Drosophila Attacin/Diptericin Gene Family. *Biochem. Biophys. Res. Commun.* **2000**, *279*, 574–581. [CrossRef] [PubMed]

59. Wang, L.-N.; Yu, B.; Han, G.-Q.; Chen, D.-W. Molecular cloning, expression in *Escherichia coli* of Attacin A gene from Drosophila and detection of biological activity. *Mol. Biol. Rep.* **2009**, *37*, 2463–2469. [CrossRef]

60. Lin, S.J.H.; Fulzele, A.; Cohen, L.B.; Bennett, E.J.; Wasserman, S.A. Bombardier Enables Delivery of Short-Form Bomanins in the Drosophila Toll Response. *Front. Immunol.* **2020**, *10*, 3040. [CrossRef]

61. Clemmons, A.W.; Lindsay, S.A.; Wasserman, S.A. An Effector Peptide Family Required for Drosophila Toll-Mediated Immunity. *PLoS Pathog.* **2015**, *11*, e1004876. [CrossRef]

62. Hanson, M.A.; Dostálová, A.; Ceroni, C.; Poidevin, M.; Kondo, S.; Lemaitre, B. Synergy and remarkable specificity of antimicrobial peptides in vivo using a systematic knockout approach. *eLife* **2019**, *8*, e44341. [CrossRef]

63. Lindsay, S.A.; Lin, S.J.; Wasserman, S.A. Short-Form Bomanins Mediate Humoral Immunity in Drosophila. *J. Innate Immun.* **2018**, *10*, 306–314. [CrossRef] [PubMed]

64. Lemaitre, B.; Kromer-Metzger, E.; Michaut, L.; Nicolas, E.; Meister, M.; Georgel, P.; Reichhart, J.M.; Hoffmann, J.A. A recessive mutation, immune deficiency (imd), defines two distinct control pathways in the Drosophila host defense. *Proc. Natl. Acad. Sci. USA* **1995**, *92*, 9465–9469. [CrossRef] [PubMed]

65. Samakovlis, C.; Kimbrell, D.A.; Kylsten, P.; Engström, A.; Hultmark, D. The immune response in Drosophila: Pattern of cecropin expression and biological activity. *EMBO J.* **1990**, *9*, 2969–2976. [CrossRef] [PubMed]

66. Tryselius, Y.; Samakovlis, C.; Kimbrell, D.A.; Hultmark, D. CecC, a cecropin gene expressed during metamorphosis in Drosophila pupae. *JBIC J. Biol. Inorg. Chem.* **1992**, *204*, 395–399. [CrossRef]

67. Tzou, P.; Reichhart, J.-M.; Lemaitre, B. Constitutive expression of a single antimicrobial peptide can restore wild-type resistance to infection in immunodeficient *Drosophila* mutants. *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 2152–2157. [CrossRef]

68. Wicker, C.; Reichhart, J.M.; Hoffmann, D.; Hultmark, D.; Samakovlis, C.; Hoffmann, J.A. Insect immunity. Characterization of a Drosophila cDNA encoding a novel member of the diptericin family of immune peptides. *J. Biol. Chem.* **1990**, *265*, 22493–22498. [CrossRef]

69. Lee, J.H.; Cho, K.S.; Lee, J.; Yoo, J.; Lee, J.; Chung, J. Diptericin-like protein: An immune response gene regulated by the anti-bacterial gene induction pathway in Drosophila. *Gene* **2001**, *271*, 233–238. [CrossRef]

70. Chiu, H.; Ring, B.C.; Sorrentino, R.P.; Kalamarz, M.; Garza, D.; Govind, S. dUbc9 negatively regulates the Toll-NF-κB pathways in larval hematopoiesis and drosomycin activation in Drosophila. *Dev. Biol.* **2005**, *288*, 60–72. [CrossRef]

71. Zhang, Z.; Zhu, S. Functional role of charged residues in drosomycin, a Drosophila antifungal peptide. *Dev. Comp. Immunol.* **2010**, *34*, 953–958. [CrossRef]

72. Ayres, J.S.; Freitag, N.; Schneider, D.S. Identification of Drosophila Mutants Altering Defense of and Endurance to *Listeria monocytogenes* Infection. *Genetics* **2008**, *178*, 1807–1815. [CrossRef]

73. Hanson, M.A.; Cohen, L.B.; Marra, A.; Iatsenko, I.; Wasserman, S.A.; Lemaitre, B. The Drosophila Baramicin polypeptide gene protects against fungal infection. *PLoS Pathog.* **2021**, *17*, e1009846. [CrossRef] [PubMed]

74. Ha, E.-M.; Oh, C.-T.; Ryu, J.-H.; Bae, Y.-S.; Kang, S.-W.; Jang, I.-H.; Brey, P.T.; Lee, W.-J. An Antioxidant System Required for Host Protection against Gut Infection in Drosophila. *Dev. Cell* **2005**, *8*, 125–132. [CrossRef] [PubMed]

75. Keebaugh, E.S.; Schlenke, T.A. Adaptive Evolution of a Novel Drosophila Lectin Induced by Parasitic Wasp Attack. *Mol. Biol. Evol.* **2011**, *29*, 565–577. [CrossRef] [PubMed]

76. Goto, A.; Yano, T.; Terashima, J.; Iwashita, S.; Oshima, Y.; Kurata, S. Cooperative Regulation of the Induction of the Novel Antibacterial Listericin by Peptidoglycan Recognition Protein LE and the JAK-STAT Pathway. *J. Biol. Chem.* **2010**, *285*, 15731–15738. [CrossRef]

77. Pal, S.; Leger, R.J.S.; Wu, L. Fungal Peptide Destruxin A Plays a Specific Role in Suppressing the Innate Immune Response in Drosophila melanogaster. *J. Biol. Chem.* **2007**, *282*, 8969–8977. [CrossRef]

78. Brun, S.; Vidal, S.; Spellman, P.; Takahashi, K.; Tricoire, H.; Lemaitre, B. The MAPKKK Mekk1 regulates the expression of Turandot stress genes in response to septic injury in Drosophila. *Genes Cells* **2006**, *11*, 397–407. [CrossRef]

79. Levashina, E.A.; Ohresser, S.; Bulet, P.; Reichhart, J.-M.; Hetru, C.; Hoffmann, J.A. Metchnikowin, a Novel Immune-Inducible Proline-Rich Peptide from Drosophila with Antibacterial and Antifungal Properties. *Eur. J. Biochem.* **1995**, *233*, 694–700. [CrossRef]

80. Levashina, E.; Ohresser, S.; Lemaitre, B.; Imler, J.-L. Two distinct pathways can control expression of the gene encoding the Drosophila antimicrobial peptide metchnikowin. *J. Mol. Biol.* **1998**, *278*, 515–527. [CrossRef]

81. Levashina, E.A.; Langley, E.; Green, C.; Gubb, D.; Ashburner, M.; Hoffmann, J.A.; Reichhart, J.-M. Constitutive Activation of Toll-Mediated Antifungal Defense in Serpin-Deficient *Drosophila*. *Science* **1999**, *285*, 1917–1919. [CrossRef]

82. Kurucz, É.; Markus, R.; Zsámboki, J.; Folkl-Medzihradszky, K.; Darula, Z.; Vilmos, P.; Udvardy, A.; Krausz, I.; Lukacsovich, T.; Gateff, E.; et al. Nimrod, a Putative Phagocytosis Receptor with EGF Repeats in Drosophila Plasmatocytes. *Curr. Biol.* **2007**, *17*, 649–654. [CrossRef]

83. Somogyi, K.; Sipos, B.; Pénzes, Z.; Andó, I. A conserved gene cluster as a putative functional unit in insect innate immunity. *FEBS Lett.* **2010**, *584*, 4375–4378. [CrossRef] [PubMed]

84. Buchon, N.; Poidevin, M.; Kwon, H.-M.; Guillou, A.; Sottas, V.; Lee, B.-L.; Lemaitre, B. A single modular serine protease integrates signals from pattern-recognition receptors upstream of the *Drosophila* Toll pathway. *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 12442–12447. [CrossRef] [PubMed]

85. Gottar, M.; Gobert, V.; Michel, T.; Belvin, M.; Duyk, G.; Hoffmann, J.A.; Ferrandon, D.; Royet, J. The Drosophila immune response against Gram-negative bacteria is mediated by a peptidoglycan recognition protein. *Nat. Cell Biol.* **2002**, *416*, 640–644. [CrossRef] [PubMed]

86. Mellroth, P.; Steiner, H. PGRP-SB1: An N-acetylmuramoyl l-alanine amidase with antibacterial activity. *Biochem. Biophys. Res. Commun.* **2006**, *350*, 994–999. [CrossRef]

87. Zaidman-Rémy, A.; Poidevin, M.; Hervé, M.; Welchman, D.P.; Paredes, J.C.; Fahlander, C.; Steiner, H.; Mengin-Lecreulx, D.; Lemaitre, B. Drosophila Immunity: Analysis of PGRP-SB1 Expression, Enzymatic Activity and Function. *PLoS ONE* **2011**, *6*, e17231. [CrossRef]

88. Bischoff, V.; Vignal, C.; Boneca, I.G.; Michel, T.; Hoffmann, J.A.; Royet, J. Function of the drosophila pattern-recognition receptor PGRP-SD in the detection of Gram-positive bacteria. *Nat. Immunol.* **2004**, *5*, 1175–1180. [CrossRef]

89. Dushay, M.S.; Asling, B.; Hultmark, D. Origins of immunity: Relish, a compound Rel-like gene in the antibacterial defense of Drosophila. *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 10343–10347. [CrossRef]

90. Hedengren, M.; Bengtsling, B.; Dushay, M.S.; Ando, I.; Ekengren, S.; Wihlborg, M.; Hultmark, D. Relish, a Central Factor in the Control of Humoral but Not Cellular Immunity in Drosophila. *Mol. Cell* **1999**, *4*, 827–837. [CrossRef]

91. Seong, C.-S.; Varela-Ramirez, A.; Tang, X.; Anchondo, B.; Magallanes, D.; Aguilera, R.J. Cloning and Characterization of a Novel Drosophila Stress Induced DNase. *PLoS ONE* **2014**, *9*, e103564. [CrossRef]

92. Castillejo-López, C.; Häcker, U. The serine protease Sp7 is expressed in blood cells and regulates the melanization reaction in Drosophila. *Biochem. Biophys. Res. Commun.* **2005**, *338*, 1075–1082. [CrossRef]

93. Dudzic, J.P.; Hanson, M.A.; Iatsenko, I.; Kondo, S.; Lemaitre, B. More Than Black or White: Melanization and Toll Share Regulatory Serine Proteases in Drosophila. *Cell Rep.* **2019**, *27*, 1050–1061.e3. [CrossRef] [PubMed]

94. Kambris, Z.; Brun, S.; Jang, I.-H.; Nam, H.-J.; Romeo, Y.; Takahashi, K.; Lee, W.-J.; Ueda, R.; Lemaitre, B. Drosophila Immunity: A Large-Scale In Vivo RNAi Screen Identifies Five Serine Proteases Required for Toll Activation. *Curr. Biol.* **2006**, *16*, 808–813. [CrossRef] [PubMed]

95. Jang, I.-H.; Chosa, N.; Kim, S.-H.; Nam, H.-J.; Lemaitre, B.; Ochiai, M.; Kambris, Z.; Brun, S.; Hashimoto, C.; Ashida, M.; et al. A Spätzle-Processing Enzyme Required for Toll Signaling Activation in Drosophila Innate Immunity. *Dev. Cell* **2006**, *10*, 45–55. [CrossRef] [PubMed]

96. Dostálová, A.; Rommelaere, S.; Poidevin, M.; Lemaitre, B. Thioester-containing proteins regulate the Toll pathway and play a role in Drosophila defence against microbial pathogens and parasitoid wasps. *BMC Biol.* **2017**, *15*, 79. [CrossRef] [PubMed]

97. Boutros, M.; Agaisse, H.; Perrimon, N. Sequential Activation of Signaling Pathways during Innate Immune Responses in Drosophila. *Dev. Cell* **2002**, *3*, 711–722. [CrossRef]

98. Zhong, W.; McClure, C.D.; Evans, C.R.; Mlynski, D.T.; Immonen, E.; Ritchie, M.G.; Priest, N.K. Immune anticipation of mating in *Drosophila*: *Turandot M* promotes immunity against sexually transmitted fungal infections. *Proc. R. Soc. B Boil. Sci.* **2013**, *280*, 20132018. [CrossRef]

99. Wang, Z.; Berkey, C.D.; Watnick, P.I. The Drosophila Protein Mustard Tailors the Innate Immune Response Activated by the Immune Deficiency Pathway. *J. Immunol.* **2012**, *188*, 3993–4000. [CrossRef]

100. Irving, P.; Troxler, L.; Heuer, T.S.; Belvin, M.; Kopczynski, C.; Reichhart, J.-M.; Hoffmann, J.A.; Hetru, C. A genome-wide analysis of immune responses in *Drosophila*. *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 15119–15124. [CrossRef]

101. Hanson, M.A.; Lemaitre, B.; Unckless, R.L. Dynamic Evolution of Antimicrobial Peptides Underscores Trade-Offs Between Immunity and Ecological Fitness. *Front. Immunol.* **2019**, *10*, 2620. [CrossRef]

102. Cao, X.; Jiang, H. Building a platform for predicting functions of serine protease-related proteins in Drosophila melanogaster and other insects. *Insect Biochem. Mol. Biol.* **2018**, *103*, 53–69. [CrossRef]

103. Ligoxygakis, P.; Pelte, N.; Hoffmann, J.A.; Reichhart, J.-M. Activation of *Drosophila* Toll During Fungal Infection by a Blood Serine Protease. *Science* **2002**, *297*, 114–116. [CrossRef]

104. Patrnogic, J.; Leclerc, V. The serine protease homolog spheroide is involved in sensing of pathogenic Gram-positive bacteria. *PLoS ONE* **2017**, *12*, e0188339. [CrossRef]

105. Kr, P.; Lee, J.; Mortimer, N.T. The S1A Protease Family Members CG10764 and CG4793 Regulate Cellular Immunity in Dro-sophila. *Micropublication Biol.* **2021**, *2021*. [CrossRef]

106. Hayden, M.S.; Ghosh, S. NF-κB in immunobiology. *Cell Res.* **2011**, *21*, 223–244. [CrossRef]

107. Hogan, P.G. Calcium–NFAT transcriptional signalling in T cell activation and T cell exhaustion. *Cell Calcium* **2017**, *63*, 66–69. [CrossRef]

108. Villarino, A.; Kanno, Y.; Ferdinand, J.R.; O'Shea, J.J. Mechanisms of Jak/STAT Signaling in Immunity and Disease. *J. Immunol.* **2014**, *194*, 21–27. [CrossRef]

109. Gallant, S.; Gilkeson, G. ETS transcription factors and regulation of immunity. *Arch. Immunol. Ther. Exp.* **2006**, *54*, 149–163. [CrossRef]

110. Copley, R.R.; Totrov, M.; Linnell, J.; Field, S.; Ragoussis, J.; Udalova, I.A. Functional conservation of Rel binding sites in drosophilid genomes. *Genome Res.* **2007**, *17*, 1327–1335. [CrossRef]

111. Immarigeon, C.; Bernat-Fabre, S.; Guillou, E.; Verger, A.; Prince, E.; Benmedjahed, M.A.; Payet, A.; Couralet, M.; Monte, D.; Villeret, V.; et al. Mediator complex subunit Med19 binds directly GATA transcription factors and is required with Med1 for GATA-driven gene regulation in vivo. *J. Biol. Chem.* **2020**, *295*, 13617–13629. [CrossRef]

112. Juven-Gershon, T.; Hsu, J.-Y.; Theisen, J.; Kadonaga, J.T. The RNA polymerase II core promoter—The gateway to transcription. *Curr. Opin. Cell Biol.* **2008**, *20*, 253–259. [CrossRef]

113. Ngoc, L.V.; Kassavetis, G.A.; Kadonaga, J.T. The RNA Polymerase II Core Promoter in *Drosophila*. *Genetics* **2019**, *212*, 13–24. [CrossRef] [PubMed]

114. Reichhart, J.M.; Georgel, P.; Meister, M.; Lemaitre, B.; Kappler, C.; Hoffmann, J.A. Expression and nuclear translocation of the rel/NF-kappa B-related morphogen dorsal during the immune response of Drosophila. *C R Acad. Sci. III* **1993**, *316*, 1218–1224. [PubMed]

115. Petersen, U.; Björklund, G.; Ip, Y.; Engström, Y. The dorsal-related immunity factor, Dif, is a sequence-specific trans-activator of Drosophila Cecropin gene expression. *EMBO J.* **1995**, *14*, 3146–3158. [CrossRef] [PubMed]

116. Petersen, U.-M.; Kadalayil, L.; Rehorn, K.-P.; Hoshizaki, D.K.; Reuter, R.; Engström, Y. Serpent regulates Drosophila immunity genes in the larval fat body through an essential GATA motif. *EMBO J.* **1999**, *18*, 4013–4022. [CrossRef]

117. Clark, R.I.; Tan, S.W.; Péan, C.B.; Roostalu, U.; Vivancos, V.; Bronda, K.; Pilátová, M.; Fu, J.; Walker, D.W.; Berdeaux, R.; et al. MEF2 Is an In Vivo Immune-Metabolic Switch. *Cell* **2013**, *155*, 435–447. [CrossRef]

118. Bajgar, A.; Kucerova, K.; Jonatova, L.; Tomcala, A.; Schneedorferova, I.; Okrouhlik, J.; Dolezal, T. Extracellular Adenosine Mediates a Systemic Metabolic Switch during Immune Response. *PLoS Biol.* **2015**, *13*, e1002135. [CrossRef]

119. Mihajlovic, Z.; Tanasic, D.; Bajgar, A.; Perez-Gomez, R.; Steffal, P.; Krejci, A. Lime is a new protein linking immunity and metabolism in Drosophila. *Dev. Biol.* **2019**, *452*, 83–94. [CrossRef]

120. DiAngelo, J.R.; Bland, M.L.; Bambina, S.; Cherry, S.; Birnbaum, M.J. The immune response attenuates growth and nutrient storage in Drosophila by reducing insulin signaling. *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 20853–20858. [CrossRef]

121. Schwenke, R.A.; Lazzaro, B.P.; Wolfner, M.F. Reproduction–Immunity Trade-Offs in Insects. *Annu. Rev. Èntomol.* **2016**, *61*, 239–256. [CrossRef]

122. Martínez, B.A.; Hoyle, R.G.; Yeudall, S.; Granade, M.E.; Harris, T.E.; Castle, J.D.; Leitinger, N.; Bland, M.L. Innate immune signaling in Drosophila shifts anabolic lipid metabolism from triglyceride storage to phospholipid synthesis to support immune function. *PLoS Genet.* **2020**, *16*, e1009192. [CrossRef]

123. Hart, B. Biological basis of the behavior of sick animals. *Neurosci. Biobehav. Rev.* **1988**, *12*, 123–137. [CrossRef]

124. Murray, M.J.; Murray, A.B. Anorexia of infection as a mechanism of host defense. *Am. J. Clin. Nutr.* **1979**, *32*, 593–596. [CrossRef] [PubMed]

125. Adamo, S.A. Parasitic suppression of feeding in the tobacco hornworm, Manduca sexta: Parallels with feeding depression after an immune challenge. *Arch. Insect Biochem. Physiol.* **2005**, *60*, 185–197. [CrossRef] [PubMed]

126. Surendran, S.; Hückesfeld, S.; Wäschle, B.; Pankratz, M.J. Pathogen induced food evasion behavior in Drosophila larvae. *J. Exp. Biol.* **2017**, *220*, 1774–1780. [CrossRef] [PubMed]

127. Ayres, J.S.; Schneider, D.S. The Role of Anorexia in Resistance and Tolerance to Infections in Drosophila. *PLoS Biol.* **2009**, *7*, e1000150. [CrossRef]

128. Sun, J.; Liu, C.; Bai, X.; Li, X.; Li, J.; Zhang, Z.; Zhang, Y.; Guo, J.; Li, Y. Drosophila FIT is a protein-specific satiety hormone essential for feeding control. *Nat. Commun.* **2017**, *8*, 14161. [CrossRef]

129. Erkosar, B.; Kolly, S.; van der Meer, J.R.; Kawecki, T.J. Adaptation to Chronic Nutritional Stress Leads to Reduced Dependence on Microbiota in *Drosophila melanogaster*. *mBio* **2017**, *8*, e01496-17. [CrossRef]

130. Lemaitre, B.; Miguel-Aliaga, I. The Digestive Tract of *Drosophila melanogaster*. *Annu. Rev. Genet.* **2013**, *47*, 377–404. [CrossRef]

131. Wang, A.; Huen, S.C.; Luan, H.H.; Yu, S.; Zhang, C.; Gallezot, J.-D.; Booth, C.J.; Medzhitov, R. Opposing Effects of Fasting Metabolism on Tissue Tolerance in Bacterial and Viral Inflammation. *Cell* **2016**, *166*, 1512–1525.e12. [CrossRef]

132. Howick, V.M.; Lazzaro, B.P. Genotype and diet shape resistance and tolerance across distinct phases of bacterial infection. *BMC Evol. Biol.* **2014**, *14*, 56. [CrossRef]

133. Coustau, C.; Carton, Y.; Nappl, A.; Shotkoski, F.; Ffrench-Constant, R. Differential induction of antibacterial transcripts in Drosophila susceptible and resistant to parasitism by *Leptopilina boulardi*. *Insect Mol. Biol.* **1996**, *5*, 167–172. [CrossRef] [PubMed]

134. Nicolas, E.; Nappi, A.J.; Lemaitre, B. Expression of antimicrobial peptide genes after infection by parasitoid wasps in Drosophila. *Dev. Comp. Immunol.* **1996**, *20*, 175–181. [CrossRef]

135. Leulier, F.; Parquet, C.; Pili-Floury, S.; Ryu, J.-H.; Caroff, M.; Lee, W.-J.; Mengin-Lecreulx, D.; Lemaitre, B. The Drosophila immune system detects bacteria through specific peptidoglycan recognition. *Nat. Immunol.* **2003**, *4*, 478–484. [CrossRef] [PubMed]

136. Kraaijeveld, A.R.; Layen, S.J.; Futerman, P.H.; Godfray, H.C.J. Lack of Phenotypic and Evolutionary Cross-Resistance against Parasitoids and Pathogens in Drosophila melanogaster. *PLoS ONE* **2012**, *7*, e53002. [CrossRef]

137. Bentz, M.L.; Humphrey, E.A.; Harshman, L.G.; Wayne, M.L. Sigma Virus (DMelSV) Incidence in Lines of Drosophila melanogaster Selected for Survival following Infection with Bacillus cereus. *Psyche A J. Èntomol.* **2017**, *2017*, e3593509. [CrossRef]

138. Cattadori, I.; Boag, B.; Hudson, P. Parasite co-infection and interaction as drivers of host heterogeneity. *Int. J. Parasitol.* **2008**, *38*, 371–380. [CrossRef]

139. Salam, N.; Mustafa, S.; Hafiz, A.; Chaudhary, A.A.; Deeba, F.; Parveen, S. Global prevalence and distribution of coinfection of malaria, dengue and chikungunya: A systematic review. *BMC Public* **2018**, *18*, 710. [CrossRef]

140. Dieme, C.; Zmarlak, N.M.; Brito-Fravallo, E.; Travaillé, C.; Pain, A.; Cherrier, F.; Genève, C.; Alvarez, E.C.; Riehle, M.M.; Vernick, K.D.; et al. Exposure of Anopheles mosquitoes to trypanosomes reduces reproductive fitness and enhances susceptibility to Plasmodium. *PLoS Negl. Trop. Dis.* **2020**, *14*, e0008059. [CrossRef]

141. Pokutnaya, D.; Molaei, G.; Weinberger, D.M.; Vossbrinck, C.R.; Diaz, A.J. Prevalence of Infection and Co-Infection and Presence of Rickettsial Endosymbionts in Ixodes scapularis (Acari: Ixodidae) in Connecticut, USA. *J. Parasitol.* **2020**, *106*, 30–37. [CrossRef]

142. Sheehan, G.; Tully, L.; Kavanagh, K.A. Candida albicans increases the pathogenicity of Staphylococcus aureus during polymicrobial infection of Galleria mellonella larvae. *Microbiology* **2020**, *166*, 375–385. [CrossRef]

143. Diaz, J.H. Tickborne Coinfections in the United States. *J. La. State Med. Soc.* **2016**, *168*, 44–53. [PubMed]

144. Markow, T.A. The secret lives of Drosophila flies. *eLife* **2015**, *4*, e06793. [CrossRef] [PubMed]

145. Small, C.; Paddibhatla, I.; Rajwani, R.; Govind, S. An Introduction to Parasitic Wasps of Drosophila and the Antiparasite Immune Response. *J. Vis. Exp.* **2012**, *63*, e3347. [CrossRef] [PubMed]

146. Galko, M.J.; Krasnow, M.A. Cellular and Genetic Analysis of Wound Healing in Drosophila Larvae. *PLoS Biol.* **2004**, *2*, e239. [CrossRef]

147. Schlamp, F.; Delbare, S.Y.N.; Early, A.; Wells, M.T.; Basu, S.; Clark, A.G. Dense time-course gene expression profiling of the Drosophila melanogaster innate immune response. *BMC Genom.* **2021**, *22*, 304. [CrossRef]

148. Senger, K.; Armstrong, G.W.; Rowell, W.; Kwan, J.M.; Markstein, M.; Levine, M. Immunity Regulatory DNAs Share Common Organizational Features in Drosophila. *Mol. Cell* **2004**, *13*, 19–32. [CrossRef]

149. Senger, K.; Harris, K.; Levine, M. GATA factors participate in tissue-specific immune responses in *Drosophila* larvae. *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 15957–15962. [CrossRef]

150. Tanji, T.; Hu, X.; Weber, A.N.R.; Ip, Y.T. Toll and IMD Pathways Synergistically Activate an Innate Immune Response in *Drosophila melanogaster*. *Mol. Cell. Biol.* **2007**, *27*, 4578–4588. [CrossRef]

151. Unckless, R.L.; Rottschaefer, S.M.; Lazzaro, B.P. The Complex Contributions of Genetics and Nutrition to Immunity in Drosophila melanogaster. *PLoS Genet.* **2015**, *11*, e1005030. [CrossRef]