Gene expression on the fly: A transcriptome-level view of *Drosophila*'s immune response to the opportunistic fungal pathogen *Aspergillus flavus*

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Abstract

Aspergilloses are opportunistic infections in animals and humans caused by several *Aspergillus* species, including *Aspergillus flavus*. Although the immune system of *Drosophila melanogaster* is extensively studied, little is known about the fly's specific responses to infection by *A. flavus*. We compared gene expression levels during induced infections in *D. melanogaster* by a virulent *A. flavus* isolate and a less virulent isolate, as well as from uninfected flies as a control. We found that 1,081 of the 14,554 gene regions detected were significantly differentially expressed among treatments. Some of these up- and down- regulated genes were previously shown to be involved in defense responses against pathogens. Some are known to be involved in vitelline membrane formation in flies. Other up- and down-regulated genes are of unknown function. Understanding expression of these genes during the process of infection in flies should improve our knowledge of innate immunity in invertebrates, and

by extension, in vertebrates as well.

Key words: Aspergillosis, *Aspergillus flavus*, *Drosophila melanogaster*, transcriptome, immune response

1. Introduction

Aspergilloses are infections caused by *Aspergillus*; in humans they are among the most hazardous mycoses and the most expensive to treat (Krueger and Nelson, 2009). *Apergillus flavus* is the second most important causal agent of human aspergilloses after *A. fumigatus*. Its conidia, asexual spores mainly dispersed by wind, can be lethal when immunocompromised patients inhale them, in some cases causing allergy, allergic bronchopulmonary aspergillosis, aspergillomas, and invasive aspergillosis (Latgé, 1999).

Drosophila melanogaster has become in a powerful model for aspergillosis studies in the last 15 years (Ben-Ami et al., 2010; Lionakis et al., 2005; Lionakis and Kontoyiannis, 2010; Ramírez-Camejo et al., 2014). Its similarity to the mammalian innate immune system makes it an attractive alternative to mammalian models to decipher host-pathogen interactions (Alarco et al., 2004; De Gregorio et al., 2001; Limmer et al., 2011). The innate immune system in *Drosophila* is the first line of defense against pathogens; it responds very quickly, but non-specifically, against invading organisms (Lemaitre and Hoffmann, 2007). Responses of insects to *Aspergillus* infections are also of interest for other reasons; for example, *Aspergillus* was proposed as a potential biological control agent of mosquitos and other pests (Ragavendran et al., 2018). On the other hand, these infections are of concern in honeybees and other beneficial insects (Foley et al., 2014). Furthermore, there is increasing concern about loss of insect diversity due to climate change, pesticide use and habitat fragmentation (Roy et al., 2009). All

these conditions make insects more susceptible to opportunistic pathogens, so understanding their defense mechanisms is important in this context.

Recently we showed that *A. flavus* isolates differ in virulence in two lines of wild-type *Drosophila melanogaster* (Ramírez-Camejo et al., 2014). This difference among isolates could be a result of differential gene expression in the fungi, of differential gene expression the fungi cause in the flies, or both. It is unclear how the innate immune response of flies varies when challenged with pathogens of different virulence levels.

The flies' defense mechanisms against pathogens are very well understood (Lemaitre and Hoffmann, 2007; Limmer et al., 2011). However, little attention has been given to exploring genes involved in the immune response against opportunistic fungal pathogens like *A. flavus*. In this study we compare gene expression levels during induced infections in *D. melanogaster* by a highly virulent *A. flavus* isolate versus a less virulent isolate (Ramírez-Camejo et al., 2014). We monitored the genome-wide expression profile of adult flies in response to fungal infection using transcriptomics. We hypothesized that the more virulent *A. flavus* isolate will induce expression of more innate defense genes in flies than the less virulent isolate.

2. Materials and methods

2.1. *Aspergillus* isolates

Two *Aspergillus flavus* isolates were used in this study; they were previously shown to differ in virulence in *Drosophila* (Ramírez-Camejo et al., 2014). The clinical isolate 139M is less virulent than the environmental isolate ABPMA1, perhaps due to repeated subculturing or length of time in culture (Cheema and Christians, 2011; Ramírez-Camejo et al., 2014).

2.2. Infection experiment

Drosophila melanogaster Canton-S flies were grouped in the following three treatments: 1) flies infected with the virulent *Aspergillus flavus* isolate ABPMA1, isolated from an air sample; 2) flies infected with the less virulent *A. flavus* isolate 139M, originally isolated from an aspergillosis patient; and 3) uninfected flies as a control (Ramírez-Camejo et al., 2012, 2014). Flies were infected with conidia using a previously described rolling assay (Lionakis and Kontoyiannis, 2010; Ramírez-Camejo et al., 2014). Thirty-five anesthetized female flies per tube were placed on a petri plate with a layer of *A. flavus* conidia and shaken for ~1min. After inoculation, each fly had $1 – 4 \times 10^5$ conidia on its exoskeleton. We collected flies two days after inoculation, before flies started to die. Every treatment was replicated 3 times.

2.3. RNA extraction

Fly weight was 10 - 25 mg per tube (for 35 flies). Flies were covered in RNAlater and preserved at - 80°C. Frozen flies were lysed and then homogenized using a POLYTRON® PT - MR 2100 (Kinematica AG) homogenizer at 12,000 rpm for ~1 min. Total mRNA extractions followed the animal tissue protocol of the RNeasy Mini Kit (Qiagen). RNA quantity and integrity was measured for the nine samples using a Qubit spectrophotometer and Bioanalyzer 2100, respectively. Final RNA mass per sample was 100 - 250 ng.

2.4. Preparation of cDNA libraries and RNA sequencing

Libraries were prepared using the Illumina TruSeq Stranded mRNA kit (low throughput protocol). In brief, this involved purification and fragmentation of mRNA, two-strand cDNA synthesis, end repair, adenylation, ligation of barcode adapters, and PCR amplification for library enrichment.

Poly(A)-containing mRNA was concentrated using oligo-dT attached magnetic beads. RNA was fragmented (250 ± 50 bp) and prepared for cDNA synthesis using reverse transcriptase and random primers. A second cDNA synthesis removed the RNA template and synthesized a replacement strand to generate ds cDNA. The cDNA fragments were then end-repaired, adenylated and ligated to Illumina adapters with barcodes. Finally, DNA fragments were selectively enriched to amplify the amount of DNA in the library. Samples were sequenced on an Illumina MiSeq Desktop Sequencer in the Sequencing & Genotyping Facility at UPR-RP. The data were deposited in the National Center for Biotechnology Information (NCBI) Short Read Archive (SRA), and are publicly available under the accession number PRJNA377735.

2.5. Quality control, mapping, assembling, and differential expression analysis

FASTQ files were unpacked and their TruSeq adapters (R1 & R2) were removed using TagCleaner (http://tagcleaner.sourceforge.net/). We also removed artificial reads and reads of low quality (Ns >5) using PrinSeq (http://prinseq.sourceforge.net/index.html); the remaining reads were assembled and mapped to the *D. melanogaster* genome (http://support.illumina.com/sequencing/sequencing_software/igenome.ilmn). TopHat2 version 2.0.11 (http://ccb.jhu.edu/software/tophat) was used for aligning RNA sequences using the Bowtie building indices from the *Drosophila* genome and the addition of minimum intron length (i=41) and maximum intron length (I=10,458) for *Drosophila* (Deutsch and Long, 1999). Also, we specified the expected distance between paired end reads using the expected mean inner distance between mate pairs (r=165) and --read-mismatches=4, --read-gap-length=4, and --read-edit-dist=4.

Cufflinks version 2.2.1 (http://cufflinks.cbcb.umd.edu/, Trapnell et al., 2012) was used to assemble transcripts, estimate their abundances, discover novel transcripts, and test for differential expression in

the three treatments in all combinations. We ran cufflinks to assemble transcripts, cuffmerge for a final transcriptome assembly, and cuffdiff to test for differential expression between treatments using default parameters. Comparison of the gene expression data between infected flies and not infected flies was performed using the Benjamini-Hochberg false discovery rate (FDR) correction of 5% (q-value = 0.05) (Benjamini and Hochberg, 1995). Genes with an adjusted p-value or FDR < 0.05 were marked as significantly differentially expressed. The expression level of a gene was normalized by calculating the number of fragments per kilobase of exon per million fragments mapped (Mortazavi et al., 2008). The R package CummeRbund version 2.28.0 (http://compbio.mit.edu/cummeRbund/) was used to visualize Cufflinks RNA-Seq output. Comparison of whole transcriptome expression between flies infected and not infected with *A. flavus* was determined by multidimensional scaling (MDS) and dendrogram using a Jensen-Shannon distance. Volcano plots were constructed to examine differentially expressed genes across treatments. Heat maps were generated using $\log_{10}$ FPKM+1 and an alpha = 0.05, which filtered multiple-testing corrected q values to determine significance.

2.6. Gene ontology analysis

The Protein Analysis Through Evolutionary Relationships (PANTHER; http://www.pantherdb.org/) classification system was used to predict the functions of the expressed genes (Mi et al., 2013). FlyBase (http://flybase.org/) was also used to predict the biological processes of the expressed genes in *D. melanogaster*.

3. Results

The two *Aspergillus flavus* isolates used here differed significantly in virulence on *Drosophila melanogaster* (Fig. 1). RNA sequencing of flies infected and not infected with *Aspergillus flavus*

generated a total of 26.7 million reads with an average length 250 bp (Table S1). Of these, ~13 million passed purity filtering standards, of which 48.7% or 6.2 million paired reads were aligned to the *D. melanogaster* genome (Table S1 and S2).

14,554 expressed gene regions were identified and represented in a multidimensional scaling and a dendrogram (Fig. 2a,b) using the treatment replicates. Both analyses showed that uninfected flies (controls) differed in gene expression from flies infected with *A. flavus*, as expected. Volcano plots showed statistical significance of differences in gene expression among treatments (Fig. 2c). Of the 1,081 gene regions that differed significantly among treatments ($p < 0.005$; $q < 0.05$), 689 unique genes were differentially expressed (as determined using $\log_{10}$ FPKM+1 and an alpha of 0.05), as visualized in the heat map (Fig. 2d). Overall, the gene regions that differed significantly in expression were distributed as follows: 1) virulent vs. less virulent, 135 gene regions, 2) less virulent vs. control, 587, and 3) virulent vs. control, 359 (Fig. 3).

These expressed genes were categorized by PANTHER based on sequence similarity to previously characterized genes, which assigned putative functions to 679 (63%) gene regions (Table 1). Thirty-seven % of gene regions did not produce significant hits in the PANTHER database and were annotated as Uncategorized Genes.

Of the 1,081 genes showing significant changes in gene expression, levels ranged from up-regulated ($\text{Log}_2 \geq 2$) to down-regulated ($\text{Log}_2 \leq -2$) (Table 2). Overall, flies infected with the less virulent *A. flavus* isolate (139M) showed more genes significantly up-regulated, and also down-regulated, than flies infected with the virulent isolate (ABPMA1) (Table 2, Fig. 2c). Also, both treatments had more up-regulated genes expressed than down-regulated genes, but fewer expressed genes in total, compared to controls (Table 2, Fig. 2c). In general, some of these down/up- regulated genes were previously shown to be involved in defense responses against pathogens and in vitelline

membrane formation in the egg (Fig. 3, $p < 0.005$; $q < 0.05$). Fifty-eight genes previously shown to be involved in defense responses to virus, bacteria, fungi, and other pathogens varied significantly in expression levels (Table 3). Fifty gene regions of unknown function differentially expressed in flies during infection with *A. flavus* are listed in Table 4.

4. Discussion

Reads were mapped in this study following the standard-throughput mapping algorithm (Trapnell et al., 2012). The low percentage of mapped reads (45% to 55 % across treatments) could be due to various factors, for instance: mapping parameters and reads which are incompatible with common downstream software (Brueffer and Saal, 2016). The *Drosophila melanogaster* genome has 17,564 genes (Brown et al., 2014), which represent 3,010 genes more that those found in our study across treatments. This indicates that our sequencing depth was sufficient to find most genes expressed in *Drosophila.* This sequencing depth is even more impressive given that some genes are only expressed in stages or conditions not found in our treatments (i.e., adult female flies raised in the lab on artificial diet).

14,554 expressed gene regions were represented with multidimensional scaling (Figure 2a) and a dendrogram (Figure 2b). Both show considerable variation among replicates of each treatment, especially the treatment with the virulent strain of *A. flavus*. This may represent different levels of infection with the fungus (which was not measured) or variation in susceptibility among individuals based on history and health (although we pooled flies to reduce this variation). Also, it is possible that the initial genes expressed are not the same in every case, and therefore the chain of responses may take different directions.

1,081 transcripts were differentially expressed in *D. melanogaster* among treatments (Fig. 2c). They corresponded to 689 gene regions that were differentially expressed in the heatmap (Fig. 2d), suggesting that the fly's immune response is complex both in terms of gene number and intensity of expression. Similarly, previous studies on *Drosophila*'s response to bacterial and fungal infection showed 400 - 1000 genes differentially expressed between infected flies and uninfected controls (Buchon et al., 2009; De Gregorio et al., 2001; Irving et al., 2001). For instance, 990 transcripts were up- or down-regulated in *Drosophila* infected with the Gram-negative bacterium *Erwinia carotovora* (Buchon et al., 2009). Most of these genes were related to defense and stem cell proliferation in the gut.

Genes expressed in *D. melanogaster* infected with *A. flavus* are predicted to be involved in diverse functions (Table 1). 253 and 341 of the 679 genes categorized by PANTHER were involved in catalytic activity and metabolic processes, respectively. The most common catalytic classes are oxidoreductases, serine-type peptidases, and translation elongation factors, while the metabolic enzymes appeared to be serine proteases, reductases, metalloproteases, and dehydrogenases (Table 1). Some of these genes have been identified in other *Drosophila* studies in response to bacterial infection and are thought to play a central role in the fly's immune response (Buchon et al., 2009; De Gregorio et al., 2001).

There were fewer genes up-regulated in flies infected with the virulent *A. flavus* strain than with the less virulent strain, and fewer genes up-regulated in both groups of infected flies than in uninfected controls, contrary to expectations and our hypothesis (Table 2). This suggests that one component of virulence may be the ability to avoid detection by the fly's immune system. Evasion of detection is a common mechanism of virulence (Collette and Lorenz, 2011).

Nevertheless, these differences in gene expression levels could also be due to the number of live flies collected after infection methods (70% of flies inoculated with the virulent isolate vs 90% of those inoculated with the less virulent isolate) (Fig. 1). Another possible explanation for the greater number of genes expressed in flies infected with the less virulent *A. flavus* isolate is that the virulent isolate invaded host tissue more quickly, inhibiting host gene expression.

4.1. Expression of known immunity genes

During fungal invasion, phagocytosis is the keystone of an early innate immune response and host defense mechanisms of *Drosophila*. Phagocytosis is the process by which fungal particles are recognized, bound to the surface of cells and internalized into a phagosome, the organelle that forms around the engulfed foreign materials (Stuart and Ezekowitz, 2008). This study identified genes related to phagocytosis in response to *A. flavus* infection in flies (Table 3). Also, we detected genes related to peptidoglycan recognition proteins (PGRPs), which are important in pathogen recognition by the innate immune system in flies (although peptidoglycan is not found in fungi). Additionally, four genes were detected that are involved in melanization, important for pigmentation and wound healing and a common element of defense mechanism in invertebrates (De Gregorio et al., 2001). Some of these genes were previously reported as involved with immune responses in *Drosophila*, but others were not (De Gregorio et al., 2001, 2002); their potential function in defense against *A. flavus* infection remains unclear and warrants further study.

When *Drosophila melanogaster* is infected with fungi, IMD or Toll pathways are activated in the fat bodies. This study found increased expression of genes involved with these pathways which are the major regulators of immune response in flies (De Gregorio et al., 2002) (Table 3) . It has been shown that flies with mutations in the Imd pathways are more susceptible to Gram-negative bacterial

infections compared to wild-type flies, while mutants in the Toll pathway are more susceptible to fungal and Gram-positive bacterial infections (Lemaitre et al., 1996; Rutschmann et al., 2002). It will be important to determine whether genes from Toll / Imd - pathway plays a role in defense against *A. flavus* by doing experiments with Toll / Imd - deficient flies.

The Toll / Imd pathways' secretions into the hemolymph lead to the up-regulation of large numbers of genes. e.g., antimicrobial peptides used by flies as defense against the invading fungal pathogens (Carpenter et al., 2009; Lemaitre and Hoffmann, 2007; Limmer et al., 2011). Previously, drosomycin and metchnikowin were recognized as the most prominent antifungal peptides in *D. melanogaster*, at least in response to the entomopathogenic fungus *Beauveria bassiana* (Lemaitre et al., 1997; Lemaitre and Hoffmann, 2007). We found significant expression of drosomycin but not metchnikowin, suggesting that the latter is not required for response to infection of flies, at least for *A. flavus* during the first two days of infection on *Drosophila*.

Others antimicrobial peptides such as attacin, cecropin, defensin, diptericin, and drosocin are very effective against bacteria (Asling et al., 1995; Bulet et al., 1993; Kylsten et al., 1990; Samakovlis et al., 1990; Wicker et al., 1990). These peptides were significantly expressed in flies infected with *A. flavus*, except for defensin (Table 3). Cecropin A1 is not only active against bacteria (Samakovlis et al., 1990), but also possesses strong antifungal activity against *Dipodascopsis uninucleata*, *Geotrichum candidum*, *Metarhizium anisopliae*, and *Saccharomyces cerevisiae* (Ekengren and Hultmark, 1999).

JNK Pathway, JAK-STAT Pathway, and other potential genes related to the immune defense in flies are listed in Table 3. The detection of these genes could play an important role during *A. flavus* infection in flies.

4.2. Expression of other genes potentially involved in immunity

In addition to genes involved in the immune response, genes for vitelline membrane formation were also expressed in flies infected with *A. flavus* (Fig. 3, $p < 0.005$; $q < 0.05$). Similarly, virgin flies infected with the Gram-negative bacterial pathogen *Providencia rettgeri* had 25 vitelline membrane transcripts up-regulated in egg-producing females (Short and Lazzaro, 2013).

The expression of vitelline membrane in females flies infected with *A. flavus* could be related to a reallocation of resources toward immune defense and away from reproduction, or it may be the result of signaling between the immune system and egg production (Short and Lazzaro, 2013). The vitelline membrane functions in recognition and binding of outside cells (that is, sperm) and after binding, is involved in signal transduction and preventing entry of additional cells. These are essential steps in fertilization but would also be useful in defense against pathogens.

For 50 genes of unknown function, expression was significantly affected by infection with *A. flavus* (Table 4). Two of these, the genes CG4269 and CG8620, were also expressed in *Drosophila* intestine in response to infection by the bacterium *Erwinia carotovora* (Buchon et al., 2009), suggesting a potential role in defense. Also the genes CG4269, CG5791, CG9928, CG13323, CG13324, and CG16836 were expressed in adult flies during microbial infection with the bacteria *Escherichia coli*, *Micrococcus luteus*, and the entomopathogenic fungus *Beauvaria bassiana* (De Gregorio et al., 2001). These results indicate that some genes known to be expressed during bacterial infection are also expressed during fungal infection.

A limiting factor in this study is that whole flies were sampled. Extraction of RNA from specific organs or cell types involved in immune response could give us more precise picture of particular genes that flies use to combat fungal infection. For example, macrophages are responsible for conidial recognition and its death. On the other hand, collecting sufficient macrophages from infected flies to extract mRNA is a considerable technical challenge. Using the entire body, as we did

here, allows us to see the interaction of genes expressed in different parts of the fly combating the infection, as occurs in nature.

5. Conclusions

The *Drosophila melanogaster* genome sequence and next-generation sequencing technology allowed us to identify genes of importance during a host-pathogen interaction. We identified many fly genes differentially expressed among *Aspergillus* infection treatments and controls. Some of these genes are unknown but others are known to be expressed after challenge with different types of pathogens, fungi included. This suggests complex mechanisms where genes commonly used by flies for other functions could be up/down regulated in response to infections by *A. flavus*. Knowing the identity of unknown genes potentially involved in the fly's defense could suggest new experiments to understand fungal virulence and provide further insight into factors that lead to susceptibility to aspergillosis and other opportunistic pathogens.

Author contributions

Luis A. Ramírez-Camejo: performed Formal analysis, Investigation, Conceptualization, Writing – original draft. **Paul Bayman**: performed Funding acquisition, Resources, Visualization, Conceptualization, Supervision, Writing – review and editing.

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Declaration of Competing Interests

The authors declare that they have no competing interests.

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Table 1. Classification of 1,081 identified genes in *Drosophila melanogaster* during infection with two *Aspergillus flavus* isolates.

Category names are shown here for 679 genes that PANTHER classification system recognized.

Table 2. Expression differential of 1,081 genes expressed in *Drosophila melanogaster* among infection treatments.

Treatments are flies infected with *Aspergillus flavus*, either the more virulent isolate (ABPMA1) or the less virulent isolate (139M). Controls are flies without infection. Undetermined genes are those expressed differentially but without log2 ratio. All differences shown are significant ($p < 0.005$) with an FDR-adjusted p value of less of 0.05.

Table 3. Genes related to the immune response of *Drosophila melanogaster* that were differentially expressed during infection with *Aspergillus flavus*.

Treatments are abbreviated as in Figure 2. P values are less than 0.005. The q value is an adjusted p value, taking in to account the false discovery rate (FDR) of less than 0.05.

Table 4. Genes of unknown function expressed in *Drosophila melanogaster* during infection with *Aspergillus flavus*.

Treatments are abbreviated as in Figure 2. P values are less than 0.005. The q value is an adjusted p value, taking in to account the false discovery rate (FDR) of less than 0.05.

Table S1. Summary of RNA-Seq reads in *Drosophila melanogaster* infected and not infected with *Aspergillus flavus*.

Table S2. Proportion of RNA-Seq reads in *Drosophila melanogaster* infected and not infected with *Aspergillus flavus*.

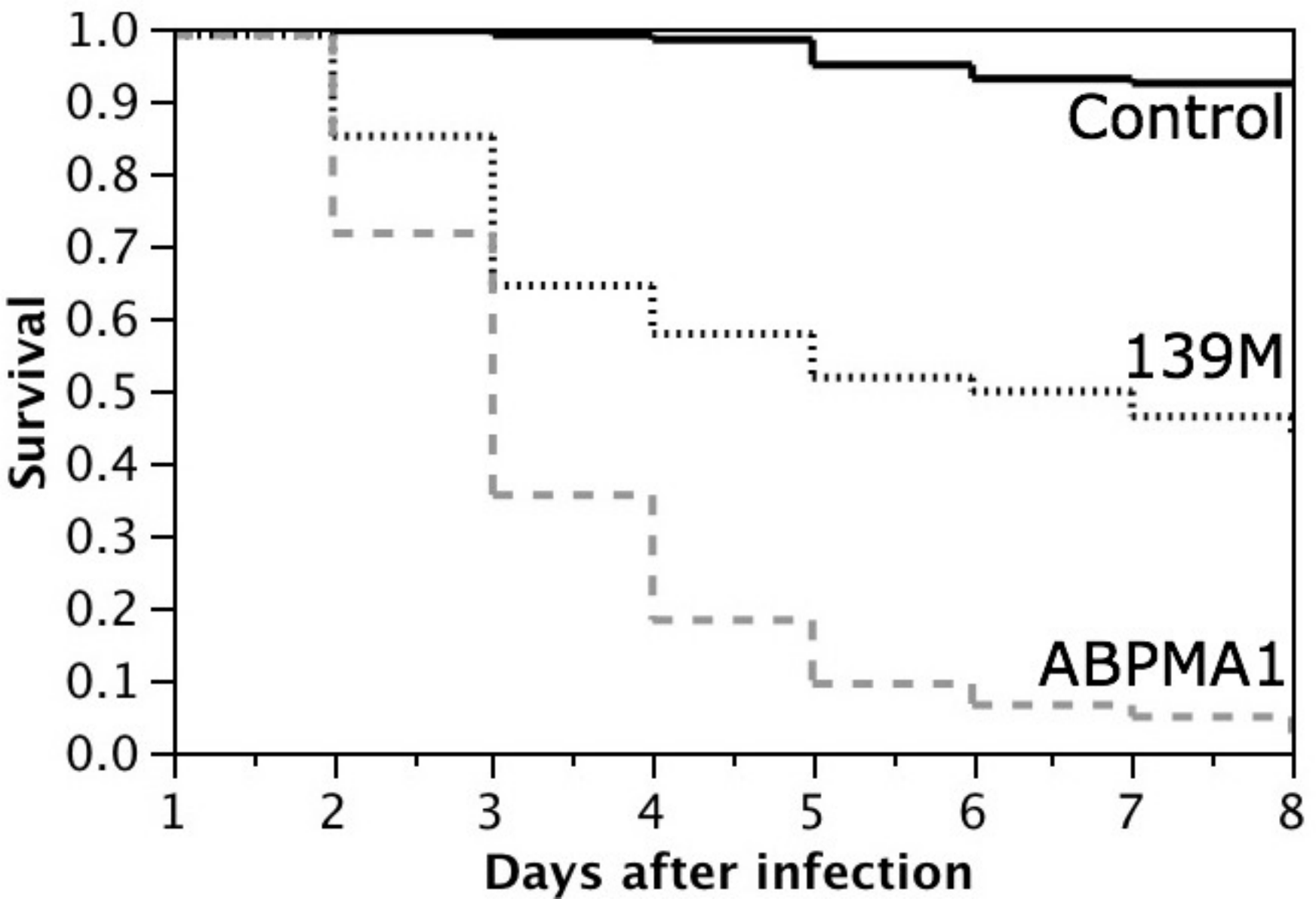
Figure 1. Virulence of *Aspergillus flavus* strains measured by and inversely proportional to survival of *Drosophila melanogaster* after infection. The y-axis shows proportion of flies surviving. Taken from Ramírez-Camejo et al., 2014.

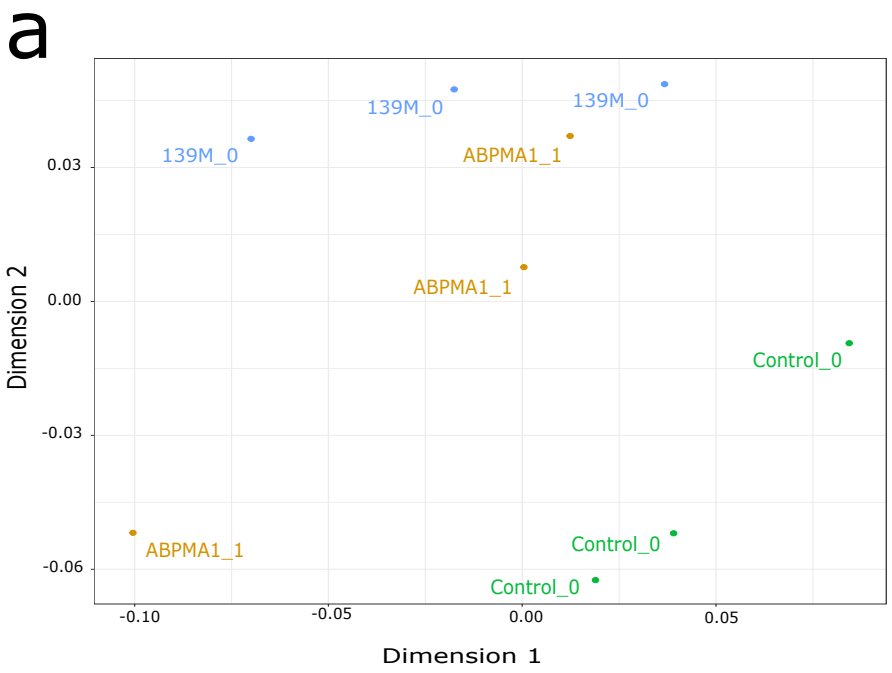
Figure 2. RNAseq analysis of *Drosophila melanogaster* infected and not infected with *Aspergillus flavus*. a) Multidimensional scaling of transcriptome data using 14,554 transcripts. Treatments are represented by colors. b) Dendrogram based on Jensen-Shannon distances of the whole transcriptome. c) Volcano plot displaying differential expressed genes between infected and not infected flies. The vertical axis (y-axis) corresponds to the mean expression value of log 10 (p-value), and the horizontal axis (x-axis) shows the $\log_2$ fold change value. Significant up-regulated expressed transcripts (right) and down-regulated expressed transcripts (left) are shown in red dots color while black dots represent genes that are not significantly differentially expressed. Positive x-values represent up-regulation and negative x-values represent down-regulation. d) Heat map of 689 differentially expressed genes in *D. melanogaster*. Color intensity represents the mean of gene expression of the Cufflinks-determined

$\log_{10}$ FPKM+1 value for three replicates in each *Drosophila* treatment [false discovery rate (FDR) < 0.05]. Treatments are abbreviated as follows: A) flies infected with less virulent *A. flavus* (139M) vs. flies infected with most virulent *A. flavus* (ABPMA1), B) flies infected with less virulent *A. flavus* (139M) vs. Control (flies without infection), and C) flies infected with most virulent *A. flavus* (ABPMA1) vs. Control (flies without infection). Figures were produced using cummerbund.

Figure 3. Venn diagram of 1,081 differentially expressed genes in *Drosophila melanogaster*. Circles are proportional to number of differentially expressed genes.

Treatments are abbreviated as seen in figure 2. The FlyBase (http://flybase.org/) and the Gene Ontology (PANTHER; http://www.pantherdb.org/) was used to display biological processes of some up/down regulated genes with $p < 0.005$ and a false discovery rate (FDR) of $q < 0.05$.


Survival
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
1
2
3
4
5
6
7
8
Days after infection
Control
139M
ABPMA1


a
139M_0
139M_0
139M_0
ABPMA1_1
ABPMA1_1
Control_0
ABPMA1_1
Control_0
Control_0
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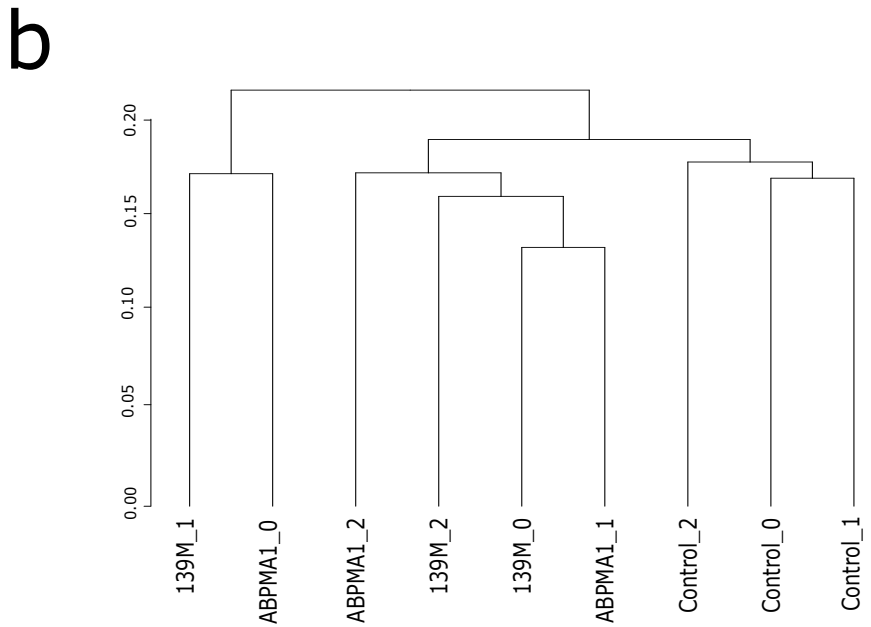
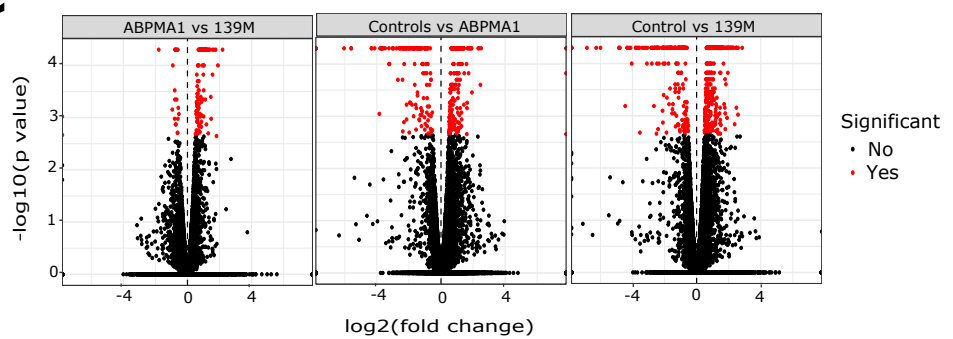
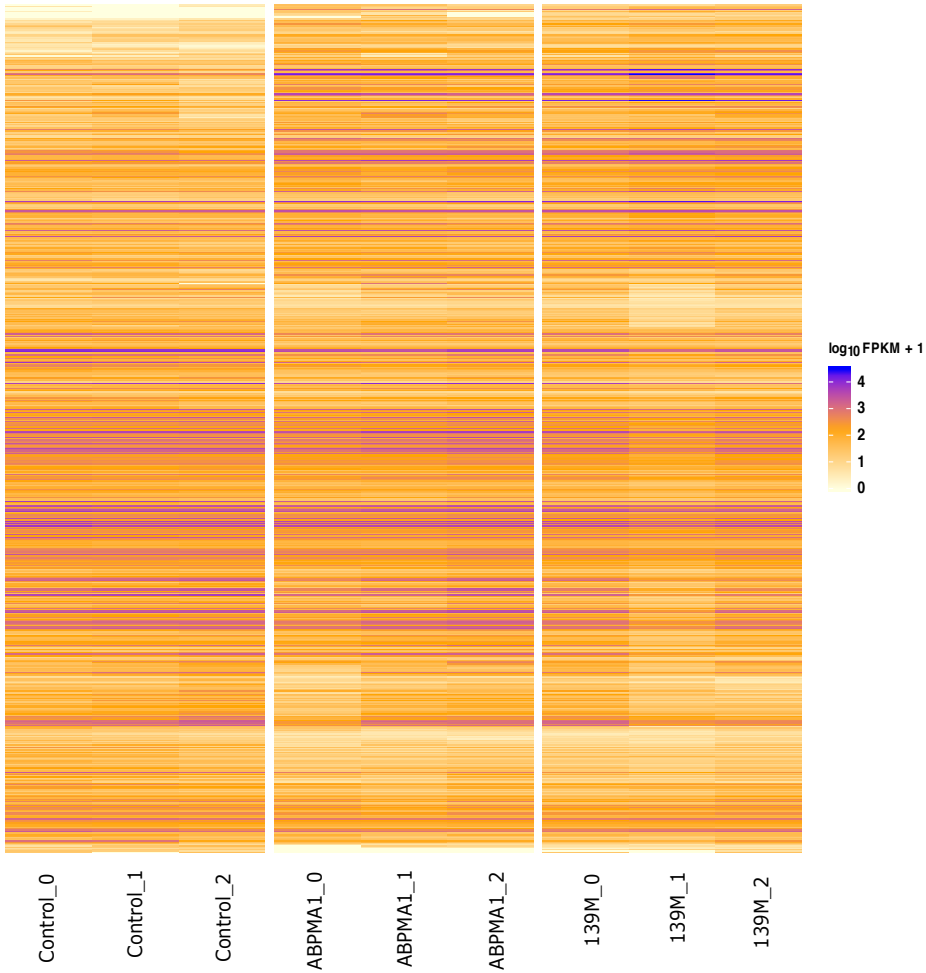
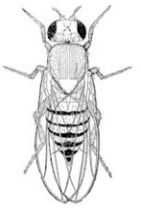
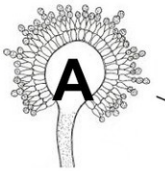
0.00
-0.03
-0.06
-0.10
-0.05
0.00
0.05
Dimension 2
Dimension 1


b
0.20
0.15
0.10
0.05
0.00
139M_1
ABPMA1_0
ABPMA1_2
139M_2
139M_0
ABPMA1_1
Control_2
Control_0
Control_1


c
ABPMA1 vs 139M
Controls vs ABPMA1
Control vs 139M
-log10(p value)
log2(fold change)
Significant
No
Yes


d
log10 FPKM + 1
Control_0
Control_1
Control_2
ABPMA1_0
ABPMA1_1
ABPMA1_2
139M_0
139M_1
139M_2





A

Up

Gene	Log2(fold_change)	Biological process
mRpS14	1.81322	Translation
Mec2	1.56519	Nephrocyte filtration
Down		
CG6639	-1.79213	Defense response and proteolysis

31

65

8

235

30

65

255

B

Up

Gene	Log2(fold_change)	Biological process
CG2070	2.4842	Metabolic process
scpr-A	2.37393	Multicellular organism reproduction
Vm32E	1.94965	Vitelline membrane formation
Cp38	1.83015	Eggshell chorion assembly
Yp2	1.7898	Vitellogenesis and others
Cp36	1.63041	Vitelline membrane formation and others
CG40486	1.5194	Metabolic process
Down		
CG12766	-3.53822	Oxidation-reduction process
CG5493	-2.6588	Oxidation-reduction process and other
CG31272	-2.08085	transmembrane transport and other
mex1	-2.05383	Embryo development and others
CG11796	-1.58767	Oxidation-reduction process and other

Up

Gene	Log2(fold_change)	Biological process
Cyp313a1	1.62402	Oxidation-reduction process
Tsp	1.61199	Muscle organ development and others
Down		
Zip3	-2.30955	Transmembrane transport and other
Zip3	-2.30955	Transmembrane transport and other

C

All genes shared

Up

Gene	Log2(fold_change)	Biological process
Yp3	A(1.2), B(2.5), C(1.3)	Vitellogenesis and others
Cpr49Ab	A(0.8), B(2.1), C(1.3)	Chitin-based cuticle development
yellow-g	A(1.1), B(1.9), C(0.8)	Melanin biosynthetic process
Cg25C	A(0.6), B(1.6), C(1.0)	Dorsal closure and other
Obp99c	A(0.6), B(1.6), C(1.0)	Sensory perception of chemical stimulus
yellow-g2	A(0.6), B(1.5), C(0.9)	Melanin biosynthetic process
CG4009	A(0.7), B(1.5), C(0.8)	Oxidation-reduction process
Down		
GstD2	A(-0.8), B(-4.0), C(-3.2)	Glutathione peroxidase activity
IM1	A(-0.8), B(-6.8), C(-6.0)	Defense response
IM2	A(-0.7), B(-4.9), C(-4.1)	Defense response
TotA	A(-0.8), B(-2.8), C(-2.0)	Defense response and others
GstE1	A(-0.7), B(-2.1), C(-1.4)	Response to oxidative stress and others
Tsf1	A(1.1), B(-1.7), C(-2.8)	Defense response and others
Dro	A(1.1), B(-1.5), C(-2.6)	Defense response and others

Shared genes between A and B

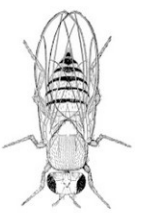
Up

Gene	Log2(fold_change)	Biological process
Vm26Ab	A(2.2), B(2.4)	Vitelline membrane formation and others
Vm26Aa	A(1.8), B(2.2)	Vitelline membrane formation
Vm34Ca	A(1.9), B(2.0)	Vitelline membrane formation
CG18110,Obp99a	A(1.2), B(1.6)	Sodium ion transport
Cp18	A(1.7), B(1.6)	Multicellular organismal development; eggshell chorion assembly
Cp15	A(1.6), B(1.5)	Multicellular organismal development; eggshell chorion assembly
CG17244	A(1.2), B(1.5)	Signal transduction.
RpL8	A(1.2), B(1.3)	Regulation of cell size and others
Down		
CG8534	A(-0.9), B(-2.3)	Fatty acid elongation
Drsl5	A(-0.9), B(-1.5)	Defense response

Shared genes between A and C

Down

Gene	Log2(fold_change)	Biological process
Dpt	A(1.4), C(-2.2)	Defense response
CecA1	A(1.5), C(-2.4)	Defense response



Shared genes between B and C

Up

Gene	Log2(fold_change)	Biological process
Obp19d	B(2.3), C(1.3)	Sensory perception of chemical stimulus
Nplp3	B(2.2), C(2.5)	Neuropeptide signaling pathway
Ance-4	B(2.2), C(1.1)	Proteolysis
Yp1	B(2.1), C(1.4)	Vitellogenesis and others
Muc11A	B(2.0), C(1.6)	Chitin metabolic process
Odc1	B(2.0), C(1.4)	Polyamine biosynthetic process
CG1678	B(2.0), C(1.7)	Imaginal disc-derived wing morphogenesis
Cpr49Ae	B(1.9), C(1.9)	Structural constituent of chitin-based cuticle
Ance-5	B(1.8), C(1.3)	Proteolysis
CG30371	B(1.7), C(1.1)	Proteolysis
pum	B(1.7), C(1.3)	Multicellular organism reproduction and others
BM-40-SPARC,Rb97	B(1.7), C(1.1)	Trachea development and others
CG33080	B(1.7), C(1.1)	Carbohydrate metabolic process
Cyp6g1	B(1.6), C(1.3)	Oxidation-reduction process and others
CG17097	B(1.6), C(1.2)	Multicellular organism reproduction and other
psd	B(1.5), C(0.9)	Vitelline membrane formation
ndl	B(1.5), C(1.1)	Eggshell chorion assembly, proteolysis and others
Obp56a	B(1.5), C(1.6)	Sensory perception of chemical stimulus
Aph-4	B(1.5), C(1.1)	Multicellular organism reproduction and others
vkg	B(1.5), C(1.2)	Malpighian tubule morphogenesis
dec-1	B(1.5), C(1.1)	Eggshell chorion assembly
Vml	B(1.5), C(0.9)	Vitelline membrane formation
CG1077	B(1.5), C(1.1)	Chorion-containing eggshell formation
Cyt-b5-r	B(1.5), C(1.1)	Oxidation-reduction process and other
Tmhs	B(1.5), C(0.8)	Sensory perception of sound
CG5162	B(1.5), C(1.3)	Multicellular organism reproduction
Down		
Arc1	B(-4.8), C(-4.3)	Response to starvation
GstD5	B(-4.8), C(-4.3)	Glutathione metabolic process
IM4	B(-4.3), C(-3.8)	Defense response and other
IM3	B(-4.3), C(-3.7)	Defense response and other
Fst	B(-3.8), C(-3.7)	Response to cold
CG13422	B(-3.1), C(-3.2)	Defense response and other
TotC	B(-2.8), C(-2.1)	Defense response and other
CG15661	B(-2.7), C(-2.2)	Metabolic process
Nep4	B(-2.6), C(-2.5)	Proteolysis
GstE7,GstE8	B(-2.6), C(-2.1)	Glutathione metabolic process
CG1698	B(-2.6), C(-2.4)	Neurotransmitter transport and other
CG10912	B(-2.5), C(-2.3)	Cold acclimation
CG30002	B(-2.5), C(-2.5)	Proteolysis
CG11893	B(-2.4), C(-2.0)	Wing disc and chaeta development
CG42825	B(-2.4), C(-2.3)	Transmembrane transport
CG13965	B(-2.4), C(-2.4)	Multicellular organism reproduction
AttA,AttB	B(-2.4), C(-2.5)	Defense response and others
CG2064	B(-2.1), C(-2.0)	Metabolic process
CG14762	B(-2.1), C(-2.0)	Neuron projection morphogenesis
CG2065	B(-2.0), C(-1.4)	Metabolic process
CG12780	B(-2.0), C(-1.9)	Defense response and other
CG10916	B(-2.0), C(-1.5)	Intracellular signal transduction and others
CG6188	B(-2.0), C(-1.6)	Response to starvation and others
CG33346	B(-1.9), C(-1.6)	Metal ion binding and others
Cyp12c1	B(-1.9), C(-1.3)	Oxidation-reduction process
CG16713	B(-1.8), C(-1.7)	Lateral inhibition
CG18067	B(-1.8), C(-2.0)	Multicellular organism reproduction
Xrp1	B(-1.8), C(-1.7)	Olfactory behavior and others
Lsp1beta	B(-1.8), C(-1.6)	Lateral inhibition
CG15067	B(-1.8), C(-1.7)	Neurogenesis.
Uro	B(-1.8), C(-1.2)	Oxidation-reduction process and others
Drs	B(-1.7), C(-1.7)	Defense response and others
Cyp6d2	B(-1.6), C(-1.2)	Oxidation-reduction process
Cyp309a1	B(-1.6), C(-1.5)	Oxidation-reduction process
CG14906,CG14907	B(-1.6), C(-1.1)	Metabolic process; methylation
CG13510,CG13511	B(-1.6), C(-1.7)	Cold acclimation
CG10477	B(-1.5), C(-1.5)	Proteolysis
Spn28Dc	B(-1.1), C(-1.8)	Defense response and others

Table 1. Classification of 1081 identified genes in *Drosophila melanogaster* during infection with two *Aspergillus flavus* isolates.

Category of Genes	Accession	# of Genes	Category of Genes	Accession	# of Genes
Uncategorized Genes		402	**Cellular Component (Continuation)**		
PANTHER Categorized Genes		679	Structural protein	PC00211	5
Molecular Function			Surfactant	PC00212	2
Antioxidant activity	GO:0016209	2	Transcription factor	PC00218	15
Binding	GO:0005488	122	Transfer/carrier protein	PC00219	11
Catalytic activity	GO:0003824	253	Transferase	PC00220	53
Enzyme regulator activity	GO:0030234	30	Transporter	PC00227	29
Nucleic acid binding transcription factor activity	GO:0001071	15	**Panther Pathway**		
Protein binding transcription factor activity	GO:0000988	3	5HT1 type receptor mediated signaling pathway	P04373	1
Receptor activity	GO:0004872	22	5HT2 type receptor mediated signaling pathway	P04374	1
Structural molecule activity	GO:0005198	62	5HT3 type receptor mediated signaling pathway	P04375	1
Translation regulator activity	GO:0045182	12	5HT4 type receptor mediated signaling pathway	P04376	1
Transporter activity	GO:0005215	36	Adenine and hypoxanthine salvage pathway	P02723	1
Biological Process			Alzheimer disease-presenilin pathway	P00004	2
Apoptotic process	GO:0006915	14	Apoptosis signaling pathway	P00006	3
Biological adhesion	GO:0022610	20	ATP synthesis	P02721	2
Biological regulation	GO:0065007	66	BMP_signaling_pathway-drosophila	P06211	1
Cellular component organization or biogenesis	GO:0071840	25	Cadherin signaling pathway	P00012	2
Cellular process	GO:0009987	101	Cytoskeletal regulation by Rho gtpase	P00016	2
Developmental process	GO:0032502	36	De novo purine biosynthesis	P02738	2
Immune system process	GO:0002376	32	De novo pyrimidine deoxyribonucleotide biosynthesis	P02739	1
Localization	GO:0051179	62	DPP-SCW_signaling_pathway	P06212	1
Metabolic process	GO:0008152	341	EGF receptor signaling pathway	P00018	1
Multicellular organismal process	GO:0032501	31	FAS signaling pathway	P00020	3
Reproduction	GO:0000003	16	Fructose galactose metabolism	P02744	2
Response to stimulus	GO:0050896	43	GBB_signaling_pathway	P06214	1
Cellular Component			General transcription regulation	P00023	1
Cell part	GO:0044464	38	Glycolysis	P00024	4
Extracellular matrix	GO:0031012	9	Huntington disease	P00029	3
Extracellular region	GO:0005576	15	Inflammation mediated by chemokine and cytokine signaling pathway	P00031	1
Macromolecular complex	GO:0032991	10	Integrin signalling pathway	P00034	4

Membrane	GO:0016020	9	Interferon-gamma signaling pathway	P00035	1
Organelle	GO:0043226	34	Ionotropic glutamate receptor pathway	P00037	1
Panther Protein Class			Metabotropic glutamate receptor group III pathway	P00039	1
Calcium-binding protein	PC00060	10	Methionine biosynthesis	P02753	1
Cell adhesion molecule	PC00069	10	Nicotinic acetylcholine receptor signaling pathway	P00044	1
Cell junction protein	PC00070	3	Ornithine degradation	P02758	1
Chaperone	PC00072	7	Parkinson disease	P00049	3
Cytoskeletal protein	PC00085	24	PDGF signaling pathway	P00047	1
Defense/immunity protein	PC00090	13	Pentose phosphate pathway	P02762	2
Enzyme modulator	PC00095	34	Proline biosynthesis	P02768	1
Extracellular matrix protein	PC00102	15	Purine metabolism	P02769	1
Hydrolase	PC00121	95	Pyrimidine Metabolism	P02771	1
Isomerase	PC00135	22	Pyruvate metabolism	P02772	2
Kinase	PC00137	7	SCW_signaling_pathway	P06216	1
Ligase	PC00142	6	Sulfate assimilation	P02778	1
Lyase	PC00144	12	TCA cycle	P00051	5
Membrane traffic protein	PC00150	6	TGF-beta signaling pathway	P00052	1
Nucleic acid binding	PC00171	63	Toll receptor signaling pathway	P00054	1
Oxidoreductase	PC00176	82	Toll_pathway_drosophila	P06217	4
Phosphatase	PC00181	7	Transcription regulation by bzip transcription factor	P00055	1
Protease	PC00190	45	Vasopressin synthesis	P04395	2
Receptor	PC00197	24	Vitamin D metabolism and pathway	P04396	1
Signaling molecule	PC00207	32	Wnt signaling pathway	P00057	2
Storage protein	PC00210	7	Xanthine and guanine salvage pathway	P02788	1

Category names are shown here for 679 genes that PANTHER classification system recognized.

Table 2. Expression differential of 1081 genes expressed in *Drosophila melanogaster* among infection treatments.

	Differential expression level						
Treatments	Up regulation (Log2 ≥ 2)	Up-moderate regulation (Log2 ≥ 1.5 & < 2)	Up-low regulation (Log2 ≥ 0.5 & < 1.5)	Down-high regulation (Log2 ≤ −2)	Down-moderate regulation (Log2 ≤ −1.5 & > −2)	Down-low regulation (Log2 ≥ −0.5 & < −1.5)	Undetermined
139M vs Control	19	38	332	58	32	101	7
ABPMA1 vs Control	4	13	188	50	24	70	10
139M vs ABPMA1	1	6	106	0	1	19	2

Treatments are flies infected with *Aspergillus flavus,* either the more virulent isolate (ABPMA1) or the less virulent isolate (139M). Controls are flies without infection. Undetermined genes are those expressed differentially but without log2 ratio. All differences shown are significant ($p < 0.005$) with an FDR-adjusted p value of less of 0.05.

Table 3. Genes related to the immune response of *Drosophila melanogaster* that were differentially expressed during infection with *Aspergillus flavus.*

Gene/Name of gene	Treatment	Log2 (fold_change)	p_value	q_value	Defense response to
Phagocytosis					
Fad2	A	-0.5336	0.0008	0.0455	---
PNUTS	B	-0.7237	5.00E-5	0.0021	---
CG5844	B	0.6034	0.002	0.0455	---
Tig/Tiggrin	B	1.0127	0.0003	0.0102	---
ATPsyn-b	B	2.7503	5.00E-5	0.0021	---
Gel/Gelsolin	B and C	B (1.0866), C (0.8305)	B - C (5.00E-5)	B - C (0.0021)	---
JNK Pathway					
Hsromega/Heat shock RNA ω	B	-1.0591	5.00E-5	0.0021	---
Taf2/TBP-associated factor 2	B	-0.6617	0.0016	0.0396	---
Gadd45	B and C	B (-1.3720), C (-1.3407)	B (9.00E-4), C (0.0012)	B (0.0248), C (0.0309)	---
JAK-STAT Pathway					
PSR/Phosphatidylserine receptor	A and B	A (0.7164), B (0.6135)	A (3.00E-4), B (0.0011)	A (0.0102), B (0.0300)	---
CG31694	B and C	B (-0.7291), C (-0.5428)	B (5.00E-5), C (0.0021)	B (0.0008), C (0.0238)	---
Toll Pathway					
wbl /Windbeutel	C	1.1424	0.0013	0.004	---
SPE/Spatzle-Processing Enzyme	C	-0.9552	5.00E-5	0.048	Bacteria and others
ndl/Nudel	B and C	B (1.5099), C (1.1286)	B - C (5.00E-5)	B - C (0.0021)	---
Dif/Dorsal-related immunity factor	B and C	B (-1.2684), C (-1.1787)	B (0.0001), C (0.0008)	B (0.0040), C (0.0228)	Bacteria, fungi, and others
Melanization					
Cp19	A and B	A (1.1129), B (1.2524)	A - B (5.00E-5)	A - B (0.0021)	---
Spn28Dc/Serpin 28Dc	B and C	B (-1.1188), C (-1.8246)	B (0.0015), C (0.00005)	B (0.0368), C (0.0021)	---
yellow-g2	A, B, and C	A (0.6278), B (1.4944), C (0.8666)	A (0.00115), B - C (5.00E-5)	A (0.03000), B - C (0.0021)	---
yellow-g	A, B, and C	A (1.1049), B (1.8638), C (0.7588)	A - C (5.00E-5)	A - C (0.00216817)	---
Imd Pathway					
Rel/Relish	B and C	B (-0.9449) C (-0.7223)	B (5.00E-5), C (0.0021)	B (0.0021), C (0.0480)	Bacteria (Gram-)
Antimicrobial peptides (AMPs)					
AttC/Attacin-C	A	1.2335	5.00E-5	0.0021	Bacteria (Gram+)
CecC/Cecropin C	C	Unknown	5.00E-5	0.0021	Bacteria (Gram+/-) and fungi
Drsl5/Drosomycin-like 5	A and B	A (-0.9120), B (-1.5229)	A (1.00E-4), B (5.00E-5)	A (0.0040), B (0.0021)	Fungi

DptB/Diptericin B	A and C	A (0.8325), C (-1.2983)	A (4.00E-4), C (5.00E-5)	A (5.00E-05), C (0.0021)	Bacteria
Dpt/Diptericin	A and C	A (1.3831), C (-2.1968)	A - C (5.00E-5)	A (5.00E-05), C (0.0021)	Bacteria
CecA1/Cecropin A1	A and C	A (1.4826), C (-2.3958)	A (0.0010), C (2.00E-4)	A - C (0.0072)	Bacteria (Gram+/-) and fungi
AttA,AttB/Attacin-A&B	B and C	B (-2.3834), C (-2.5457)	B - C (5.00E-5)	B - C (0.0021)	Bacteria (Gram+/-)
Drs/Drosomycin	B and C	B (-1.6669), C (-1.6823)	B - C (5.00E-5)	B - C (0.0021)	Bacteria, fungi, and protozoan
Dro/Drosocin	A, B, and C	A (1.1169), B (-1.5027), C (-2.6196)	A and C (5.00E-5), B (0.0007)	A and C (0.0021), B (0.0216)	Bacteria (Gram+/-)
Others					
CG6639/SPH93	A	-1.7921	5.00E-5	0.0021	Bacteria (Gram+)
PGRP-SC2	A	1.0123	3.00E-4	0.0102	---
CG9989/Stress induced DNase	A	1.2949	0.002	0.0455	Bacteria
Tep4/Thioester-containing protein 4	B	-0.8669	5.00E-5	0.0021	Bacteria
CSN8/COP9 signalosome subunit 8	B	-0.7766	0.0019	0.0446	Bacteria
spen/Split ends	B	-0.6062	5.00E-5	0.0021	Fungi
vir-1/Virus-induced RNA 1	B	0.7342	5.00E-5	0.0021	Virus
nimB2/Nimrod B2	B	0.7639	4.00E-4	0.013	Bacteria
RpS6/Ribosomal protein S6	B	0.823	5.00E-5	0.0021	---
Spn38F	B	0.9399	0.0005	0.0168	Bacteria (Gram-)
GNBP3/Gram-negative bacteria binding protein 3	B	1.0226	5.00E-4	0.0156	Fungi
Mst57Da/Male-specific RNA 57Da	B	1.164	0.0013	0.033	---
PGRP-SB1/PGRP-SB1	C	-0.838	0.0006	0.0192	---
CG13422/GNBP-like3	B and C	B (-3.0683), C (-3.2453)	B - C (5.00E-5)	B - C (0.0021)	Bacteria (Gram-)
TotC/Turandot C	B and C	B (-2.7920), C (-2.1339)	B - C (5.00E-5)	B - C (0.0021)	Bacteria
CG12780	B and C	B (-1.9950), C (-1.9127)	B - C (5.00E-5)	B - C (0.0021)	Virus
Thor/Thor	B and C	B (-0.7224), C (-0.6211)	B (5.00E-5), C (2.00E-4)	B - C (0.0021)	Bacteria
emp/Epithelial membrane protein	B and C	B (0.6280), C (0.5989)	B (0.0007), C (0.0015)	B (0.0216), C (0.0376)	---
LanA/Laminin A	B and C	B (0.7829), C (0.9590)	B - C (5.00E-5)	B - C (0.0021)	---
Anp/Andropin	B and C	B (0.8865), C (0.8513)	B (0.0003), C (0.0014)	B (0.0116), C (0.0358)	Bacteria (Gram+/-)
CG2736	B and C	B (1.1516), C (0.7502)	B (5.00E-5), C (6.00E-4)	B (0.0021), C (0.0180)	---
modSP/Modular serine protease	B and C	B (1.2213), C (1.7244)	B (6.00E-4), C (0.0001)	B (0.0180), C (0.0056)	---
CG15065	B and C	B (unknown), C (unknown)	B - C (5.00E-5)	B - C (0.0021)	---
IM3/Immune induced molecule 3	B and C	B (-4.3151), C (-3.7433)	B - C (5.00E-5)	B - C (0.0021)	Bacteria/Toll signaling pathway
IM4/Immune induced molecule 4	B and C	B (-4.3403), C (-3.7516)	B - C (5.00E-5)	B - C (0.0021)	---

IM1/Immune induced molecule 1	A, B, and C	A (-0.7939), B (-6.8414), C (-6.0474)	A (3.00E-4), B (5.00E-5), C (-6.0474)	A (0.0102), B - C (0.0021)	---
IM2/Immune induced molecule 2	A, B, and C	A (-0.7174), B (-4.8556), C (-4.1382)	A (0.00135), B - C (5.00E-5)	A (0.0339), B - C (0.0021)	---
TotA/Turandot A	A, B, and C	A (-0.7842), B (-2.8346), (-2.0503)	A (0.0004), B - C (5.00E-5)	A (0.0142), B - C (0.0021)	Bacteria
Tsf1/Transferrin 1	A, B, and C	A (1.0783), B (-1.6983), C (-2.776)	A - C (5.00E-5)	A - C (0.0021)	---

Treatments are abbreviated as in Figure 2. P values are less than 0.005. The q value is an adjusted p value, taking in to account the false discovery rate (FDR) of less than 0.05.

Table 4. Genes of unknown function expressed in *Drosophila melanogaster* during infection with *Aspergillus flavus*.

	Gene	Treatment	log2(fold_change)	p_value	q_value
1	CG18745	B	-2.5029	0.0022	0.0489
2	CG42365	B	-1.8766	0.0021	0.0472
3	CG32368	B	-1.7168	0.0006	0.0180
4	CG12310, CG18581, CG43679	B	-1.5262	0.0009	0.0249
5	CG34331	B	1.4823	0.0022	0.0489
6	CG15201	B	1.7623	0.0007	0.0204
7	CG32647	B	1.8512	0.00005	0.0022
8	CG13947	B	2.5838	0.00095	0.0259
9	CG34176	C	-1.9476	0.00055	0.0168
10	CG43774	C	-1.8075	0.0006	0.0180
11	fit	A, B, and C	A (-0.6875), B (-2.4598), C (-1.7723)	A - C (5.00E-5)	A (0.019), B - C (0.0021)
12	CG11672	A, B, and C	A (-0.6707), B (-2.0368), C (-1.3660)	A - C (5.00E-5)	A (0.020), B - C (0.0021)
13	CG34291	A, B, and C	A (-0.6014), B (-1.6211), C (-1.0197)	A - C (5.00E-5)	A - C (0.0021)
14	CG42369	A, B, and C	A (0.7133), B (1.9896), C (1.2762)	A (0.00145), B - C (5.00E-5)	A (0.035), B - C (0.0021)
15	CG16826	A, B, and C	A (1.0228), B (2.8464), C (1.8236)	A - C (5.00E-5)	A - C (0.0021)
16	CG10332	A, B, and C	A (1.3729), B (-1.6565), C (-3.0294)	A and C (5.00E-5), B (0.0003)	A (0.0040), B (0.010), C (0.0087)
17	Fcp3C	A and B	A (0.8677), B (1.4987)	A - B (5.00E-5)	A - B (0.0021)
18	CG30080, CG42662	B and C	B (-5.9942), C (-5.5992)	B - C (5.00E-5)	B (0.0021), C (0.042)
19	CG8620	B and C	B (-4.4496), C (-3.8434)	B (0.00065), C (0.0009)	B (0.019), C (0.0021)
20	CG5791	B and C	B (-4.2135), C (-3.6692)	B - C (5.00E-5)	B (0.0021), C (0.015)
21	CG15784	B and C	B (-3.6953), C (-3.5892)	B - C (5.00E-5)	B - C (0.0021)
22	CG13324	B and C	B (-3.6127), C (-3.6780)	B - C (5.00E-5)	B (0.0021), C (0.040)
23	Arc2	B and C	B (-3.3888), C (-2.965)	B - C (5.00E-5)	B - C (0.0021)
24	CG16772	B and C	B (-3.2652), C (-3.6620)	B - C (5.00E-5)	B - C (0.0021)
25	CG15423	B and C	B (-3.1013), C (-3.2118)	B - C (0.0001)	B - C (0.0040)
26	CG5550	B and C	B (-2.7905), C (-2.6873)	B (0.0001), C (0.0002)	B (0.0040), C (0.0072)
27	CG16836	B and C	B (-2.7429), C (-2.3734)	B - C (5.00E-5)	B - C (0.0021)
28	CR44035	B and C	B (-2.6145), C (-1.9102)	B (0.00005), C (0.00055)	B - C (0.0021)
29	CG12868	B and C	B (-2.5081), C (-2.2459)	B - C (5.00E-5)	B - C (0.0021)
30	CG13323	B and C	B (-2.4466), C (-2.3621)	B - C (5.00E-5)	B (0.0021), C (0.0216)
31	CG4269	B and C	B (-2.3198), C (-2.2998)	B - C (5.00E-5)	B - C (0.0021)
32	CG9928	B and C	B (-2.1415), C (-1.8839)	B - C (5.00E-5)	B - C (0.0021)
33	CG34054	B and C	B (-2.1033), C (-2.1479)	B (0.0013), C (0.0011)	B (0.0339), C (0.0021)
34	CG15044	B and C	B (-2.0810), C (-1.5515)	B (0.00005), C (0.0018)	B - C (0.0021)
35	CG15043	B and C	B (-2.0748), C (-2.1644)	B - C (5.00E-5)	B - C (0.0021)
36	CG43351	B and C	B (-1.9096), C (-1.4658)	B (0.00005), C (0.0003)	B (0.0021), C (0.0339)

37	CG30154	B and C	B (-1.8512), C (-1.7053)	B (0.0004), C (0.001)	B (0.0142), C (0.0021)
38	CG5778	B and C	B (-1.7763), C (-1.6052)	B - C (5.00E-5)	B (0.0021), C (0.01425)
39	CG4377	B and C	B (-1.6610), C (-1.4552)	B - C (5.00E-5)	B - C (0.0021)
40	CG5399	B and C	B (-1.6230), C (-1.5038)	B - C (5.00E-5)	B - C (0.0021)
41	CG10911	B and C	B (-1.6158), C (-1.4166)	B - C (5.00E-5)	B (0.0021), C (0.0168)
42	CG16775	B and C	B (-1.0421), C (-1.5056)	B - C (5.00E-5)	B (0.0021), C (0.0405)
43	CG5080	B and C	B (1.5114), C (1.0830)	B - C (0.00015)	B (0.0021), C (0.0056)
44	CG14629	B and C	B (1.5202), C (1.0617)	B - C (5.00E-5)	B - C (0.0021)
45	CG33109	B and C	B (1.7252), C (1.9113)	B - C (5.00E-5)	B - C (0.0021)
46	CG17325	B and C	B (1.7290), C (1.7463)	B - C (5.00E-5)	B - C (0.0021)
47	Msr-110	B and C	B (1.7789), C (1.1841)	B (0.00005), C (0.0001)	B (0.0021), C (0.0204)
48	CG1648	B and C	B (2.2556), C (1.2966)	B - C (5.00E-5)	B (0.0021), C (0.0278)
49	CG7203	B and C	B (2.4875), C (2.3762)	B - C (5.00E-5)	B - C (0.0021)
50	CG4000	B and C	B (2.5900), C (2.3270)	B - C (5.00E-5)	B - C (0.0021)

Treatments are abbreviated as in Figure 2. P values are less than 0.005. The q value is an adjusted p value, taking in to account the false discovery rate (FDR) of less than 0.05.