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# Transcriptional response of laboratory-reared Mexican fruit flies (*Anastrepha ludens* Loew) to desiccation

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## Abstract

Confronting environments with low relative humidity is one of the main challenges faced by insects with expanding distribution ranges. *Anastrepha ludens* (the Mexican fruit fly) has evolved to cope with the variable conditions encountered during its lifetime, which allows it to colonise a wide range of environments. However, our understanding of the mechanisms underpinning the ability of this species to confront environments with low relative humidity is incomplete. In this sense, omic approaches such as transcriptomics can be helpful for advancing our knowledge on how this species copes with desiccation stress. Considering this, in this study, we performed transcriptomic analyses to compare the molecular responses of laboratory-reared *A. ludens* exposed and unexposed to desiccation. Data from the transcriptome analyses indicated that the responses to desiccation are shared by both sexes. We identified the up-regulation of transcripts encoding proteins involved in lipid metabolism and membrane remodelling, as well as proteases and cuticular proteins. Our results provide a framework for understanding the response to desiccation stress in one of the most invasive fruit fly species in the world.

## Introduction

Desiccation resistance is a phenomenon whereby certain insects have the capability to tolerate water deprivation (Hoffmann and Parsons, 1989; Rajpurohit *et al.*, 2013; Wang *et al.*, 2021). This trait is crucial in invasive insect species that quickly adapt to environmental stress, such as fruit flies (Weldon *et al.*, 2016). The three essential mechanisms by which insects can increase desiccation resistance have been classified as: (1) reducing the rate of water lost (e.g. desiccation resistance), (2) increasing bulk water (Canteen hypothesis), and (3) tolerating greater amounts of water loss (e.g. desiccation tolerance) (Telonis-Scott *et al.*, 2006 and references there in). In *Drosophila*, the primary mechanism for surviving desiccation is increased water retention (Telonis-Scott *et al.*, 2006). In insects, water can be lost through the epicuticle, gut epithelial cells, and respiration through the spiracle (Ferveur *et al.*, 2018). The main mechanism for water retention is related to an increased volume of water contained in the haemolymph. Correlatively, desiccation-resistant flies are larger-bodied and contain a greater volume of water (Rajpurohit *et al.*, 2013; Kalra and Parkash 2014; Tejeda *et al.*, 2014). It has been previously noted that there are differences between male and female flies when exposed to desiccation (Hoffmann and Harshman, 1999; Tejeda *et al.*, 2014). Part of these differences in desiccation resistance have been attributed to the greater body volume of females compared to males, which results in a greater volume of haemolymph that contains an overall greater quantity of water compared to males (Folk *et al.*, 2001; Bazinet *et al.*, 2010; Tejeda *et al.*, 2014). In addition, several molecular mechanisms have been found to be involved in desiccation resistance, including cuticular hydrocarbon impingement, cAMP-dependent signalling of protein DESI, and carbohydrate metabolism regulation involving increased trehalose deposition (Kawano *et al.*, 2010; Thorat *et al.*, 2012; Chung *et al.*, 2014; Stinziano *et al.*, 2015). Other specialised proteins involved in water transport have been associated with water stress response in invertebrates. This is the case of Aquaporins, which are involved in the process of water retention, cryoprotection, and anhydrobiosis acting as ubiquitous membrane channels whose primary function is to facilitate the passive transport of water across the plasma membrane of the cell in response to osmotic gradients that are created by the active transport of solutes (Campbell *et al.*, 2008; Verkman *et al.*, 2014). Aquaporins have been linked to desiccation resistance in *Drosophila melanogaster* larvae (Philip *et al.*, 2008). Another interesting

process linked to desiccation resistance is protein ubiquitination, which is also an important component in the regulation of the cuticle and cytoskeleton in flies (Kang *et al.*, 2016).

*Anastrepha ludens* is one of the most invasive tephritids, whose distribution includes Central America, Mexico, and the Southern United States (USDA 2023). *Anastrepha ludens* differs from other members of *Anastrepha* in that it is not only tropical but also subtropical (Norrbom *et al.*, 1999). Its primary hosts include citrus and mango, but it also infests dozens of other fruits, and thus causes substantial financial damages each year (USDA, 2023). This species has a remarkable behavioural plasticity and adaptable physiological responses and can become tolerant to desiccation by the seventh generation without losing mating compatibility with other populations (Tejeda *et al.*, 2014, 2017). Thus, understanding the mechanisms of desiccation resistance in *A. ludens* may allow the development of new treatments to control their populations and to improve those currently in use.

While the transcriptomic response to desiccation has been previously studied in various *Drosophila* species, it has not been characterised in the Mexican fruit fly, *A. ludens* (Sinclair *et al.*, 2007; Matzkin and Markow, 2009; Wang *et al.*, 2021). *Anastrepha ludens* differs from *D. melanogaster* morphologically, physiologically, and reproductively. For example, *D. melanogaster* has an approximate lifespan of 50 days at optimal growth conditions, females lay about 400 eggs in their lifetime and are approximately 2.5 mm in length, with males being slightly smaller, and it has four pairs of chromosomes (Linford *et al.*, 2013). In contrast, *A. ludens* can live up to 1 year in the wild and lay up to 2000 eggs during its lifespan, females are 7–11 mm long, with males being slightly smaller (Carey *et al.*, 2005; Tejeda *et al.*, 2014), and it has 12 acrocentric chromosomes, including five pairs of autosomes and an XX/XY sex chromosome pair (Garcia-Martinez *et al.*, 2009).

Desiccation resistance in *D. melanogaster* has been shown to be highly heritable and possess the ability to rapidly evolve (Kellermann *et al.*, 2009). The rearing environment has also been shown to play an important role in the evolution and heritability of various *Drosophila* species. However, little is known concerning the molecular regulation of desiccation resistance in *A. ludens*. In this study, we compared the whole transcriptomic profile of two groups, exposed and unexposed to desiccation stress, of laboratory-mass-reared male and female *A. ludens*. This allowed us to identify a series of genes putatively involved in the response to desiccation stress.

## Materials and methods

### Origin of A. ludens flies

*Anastrepha ludens* flies were obtained as pupae from a mass-reared strain produced at the MOSCAFRUT biofactory in Metapa de Domínguez, Chiapas, Mexico. More than 300 million sterile flies are produced on a weekly basis. This laboratory strain had been reared for more than 100 generations in the biofactory when the study was performed. Females oviposit in an artificial medium, and their larvae develop on an artificial diet of corn cob fractions, corn flour, sodium benzoate, methylparaben, citric acid, guar gum, and purified water (Orozco-Dávila and Quintero-Fong, 2015). From this original population, we derived five experimental populations of 400 flies each that were maintained under similar conditions in the laboratory (see Tejeda *et al.*, 2016 for details of the process). These flies were placed in Plexiglas cages of 25 × 25 × 25 cm for 8 h to allow them to complete wing expansion and tanning of the body cuticle (Bochicchio *et al.*, 2021). After this period, flies were exposed to the two treatments (control or desiccation) for 24 h (see Tejeda *et al.*, 2016). The duration of the stress period was established based on previous experimental results (Tejeda *et al.*, 2016). Flies of both sexes were put in separate cages; 20 individuals were taken from each of the five populations to have 200 females and 200 males per replicate. Desiccation conditions (20–30% relative humidity) were achieved by placing in each cage three plastic containers with 50 g of silica gel each (Sigma-Aldrich, PubChem Substance ID: 24899758, Darmstadt, Germany) and covered with a nylon mesh to avoid direct contact between the flies and the silica gel (Tejeda *et al.*, 2014). All the cages were then individually sealed with a single layer of self-adhesive plastic film. Relative humidity inside the cage stabilised to 20–30% within the first 12 h of exposure. Control samples were not subjected to desiccation stress. A data logger was placed inside a cage selected at random.

### RNA sequencing analysis

RNA-Seq experiments were performed on a cohort of flies from the control and treated (exposed to desiccation for 24 h) groups. For this, two groups of five females and two groups of five males from both experimental groups were randomly formed, ultrafrozen in liquid nitrogen, and kept at −80°C until use.

For the RNA extraction, we ground the samples with a mortar and pestle with liquid nitrogen and the macerate of each sample was used for total RNA extraction. For this, we used 100 mg of the powder resulting from each pulverised pool and extracted the RNA following the standard TRI Reagent™ (Thermo Fisher Scientific) protocol. RNA integrity was checked using 1% agarose gel. In addition, the RNA integrity number (RIN) was estimated using the Agilent Bioanalyzer 2100 system (Agilent Technologies®) equipped with an Agilent® RNA 6000 Nano. RNA samples with RINs greater than 8 were sequenced.

Library construction and sequencing were carried out at the Advanced Genomics Unit of CINVESTAV-Irapuato (Guanajuato, Mexico). The sequencing libraries were constructed using the TruSeq mRNA Sample Preparation kit (Illumina®). The quantification of the products was performed using an Agilent® DNA High Sensitivity Chip on an Agilent Bioanalyzer 2100® System, a Qubit® fluorometer, and an Invitrogen from a Thermo Fisher Scientific Qubit double-stranded (ds)DNA HS Assay® kit. Libraries were sequenced on an Illumina HiSeq 4000 platform® in a paired-end 2 × 100 format.

### Bioinformatic analysis

After sequencing, we obtained two libraries for each sex (male and female) per condition (control and stressful). Raw reads were checked for quality with FastQC v0.11.3 (Andrews, 2010). The Trimmomatic v0.36 toolkit was used to trim adapters, remove ambiguous sequences (N), and remove low-quality bases (Bolger *et al.*, 2014) using the following parameters: CROP = 15, SLIDINGWINDOW = 4:28, and MINLEN = 50. Cleaned reads were checked again with FastQC. The forward and reverse reads of all libraries were concatenated in cat_R1 and cat_R2 files, respectively, and assembled using the Trinity v2.0.6 (Grabherr *et al.*, 2011) assembler. To estimate the completeness of the assembled transcriptome, we passed it on to the BUSCO

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(Benchmarking Universal Single-Copy Orthologs) program (Simão *et al.*, 2015) using the gVolante website (Nishimura *et al.*, 2017). The BUSCO metrics were accompanied by general assembly statistics computed using the Trinity package's utilities.

All the subsequent statistical analyses were performed using the utilities of the Trinity workflow. To estimate transcript expression abundances, for instance, we independently pseudoaligned all the libraries against the *de novo* transcriptome assembly using the Kallisto software (Bray *et al.*, 2016). The results were a matrix of gene raw counts, which was normalised to transcripts per million (TPM) representing 'gene' expression levels. The EdgeR 2.14 package (Robinson *et al.*, 2010) was used to identify differentially expressed genes (DEGs) between experimental conditions and for each sex. We then extracted the transcripts with the most significant False Discovery Rate (FDR) and fold changes (FC $\geq$2 or $\leq$−2 and a *P*-adjusted FDR <0.001) and clustered the transcripts according to their differential expression patterns across samples. For this, we first performed a Pearson correlation analysis to check the clustering pattern of the datasets created based on the abundances of DEGs. We also partitioned the heatmap into gene clusters with similar expression patterns to identify clusters of DEGs among treatments. The identified DEG clusters were annotated against the *Tephritidae* nucleotide sequences available on RefSeq (NCBI) (taxid: 7211) using Blastn with an *e*-value <0.000001.

## Results

The sequencing produced $16.7 \pm 2.8 \times 10^6$ (mean ± SD) reads per library, adding up to more than $533 \times 10^6$ paired reads. After cleaning, we recovered $14.4 \pm 2.6 \times 10^6$ (mean ± SD) (~86% of the total raw reads) high-quality reads equivalent to $\sim 461 \times 10^6$ clean paired reads. Raw data were deposited in the NCBI under the Bioproject PRJNA1050629.

Clean paired reads were assembled *de novo* into 126,919 transcripts containing 40,508 unigenes with a TPM value >1. The general statistics of the clean transcriptome assembly (those with a TPM value >1) can be found in fig. 1. The N50 length of the unigenes was 2293, the GC content was 38.86%, and the level of completeness was 98.72% as measured by BUSCO, indicating an acceptable level of assembly completeness.

The Pearson correlation analysis showed a high correspondence within the libraries of each experimental group, as they were grouped based on the clustering analysis of differentially expressed unigenes (fig. 1A). In the differential expression analysis, we found a clear separation among comparison groups and detected a total of 450 DEGs, which formed clear differential expression clusters (figs 1B and 2). According to the cut-clustering algorithm, we identified five representative expression clusters: cluster 1 (formed by 71 DEGs), cluster 2 (354 DEGs), cluster 3 (6 DEGs), cluster 4 (3 DEGs), cluster 4 (10 DEGs), and cluster 5 (9 DEGs). Cluster 1 represents the genes induced in both sexes in the response to stress; cluster 2 represents the DEGs with differential expression patterns between sexes; cluster 3 contains the DEGs down-regulated in treated males and up-regulated in treated females; cluster 4 represents the DEGs down-regulated in treated samples; and cluster 5 represents the unigenes induced only in treated females (not shown in fig. 3). Supplementary table 1 shows the 162 DEGs that were annotated and the cluster they belong to.

Based on the primary focus of our study, we consider clusters 1, 3, 4, and 5 to be the most interesting ones. Table 1 summarises the list of annotated DEGs of each cluster with a known function, excluding all the DEGs annotated as 'uncharacterised'.

## Discussion

Low relative humidity is an important environmental stressor that can drive the physiological and behavioural activity of insects, their geographical distributions, and their demographic dynamics (Hoffmann *et al.*, 2003). This factor has been scarcely studied in true fruit flies and their molecular responses to this condition are poorly understood. Given the potential importance of water stress in determining the invasiveness and dispersal potential of a highly pestiferous species such as *A. ludens*, in the present study we surveyed its transcriptional response under controlled desiccation conditions.

Our results indicate that the response to water stress is very similar in both males and females despite a visible size dimorphism (females are larger than males) but also differences in their lipid and water reserves (Tejeda *et al.*, 2014, 2016). In this sense, we observed the up-regulation of pancreatic lipase-related protein 2 and intermembrane lipid transfer protein Vps13, which are two important players in the regulation of lipid metabolism. Like pancreatic triglyceride lipase, pancreatic lipase-related protein 2 cleaves triglycerides but has a broader substrate specificity, since it also hydrolyses phospholipids and galactolipids (Lowe, 2000). This function is important from a dietary point of view, as galactolipids are the main components of plant membrane lipids, including fruit tissues (Sias *et al.*, 2004; Sahaka *et al.*, 2020). Vps13 proteins are evolutionarily conserved proteins that mediate the transfer of lipids between membranes at organelle contact sites and can bind a wide range of phospholipids (Park and Neiman, 2012; Kumar *et al.*, 2018; Kolakowski *et al.*, 2021). They are also involved in mitochondrial lipid homeostasis and the organisation of the actin cytoskeleton (Rzepnikowska *et al.*, 2017). In relation to this, we found that SERAC1 transcripts, required for phosphatidylglycerol remodelling, are up-regulated. Phosphatidylglycerol is involved in the biosynthesis of cardiolipin, an important constituent of the inner mitochondrial membrane. For this reason, phosphatidylglycerol remodelling is essential for mitochondrial function and intracellular cholesterol trafficking (Wortmann *et al.*, 2012). Similarly, we found that the transcript encoding phosphatidate phosphatase, LPIN3, is also up-regulated. Phosphatidate phosphatases are enzymes that catalyse the dephosphorylation of phosphatidate, producing diacylglycerol and inorganic phosphate (Carman and Han, 2006). This conversion is required for the biosynthesis of membrane phospholipids and the storage of fats, while diacylglycerol and inorganic phosphate also serve as cell-signalling molecules (Lehmann, 2021). Phosphatidate phosphatases are recognised as central regulators of the function of adipose tissue in *D. melanogaster* and are up-regulated under starvation conditions to promote survival (Ugrankar *et al.*, 2011). These results are also consistent with the induction of the transcripts encoding for Rho guanine nucleotide exchange factor 7. This protein belongs to the small GTPases of the Rho (Ras homologous) family, a protein family involved in the regulation of many cellular processes, including actin remodelling and phospholipid metabolism (Schmidt and Hall, 2002; Schmidt and Debant, 2014).

Transcript up-regulation suggests the onset of enzymes involved in lipid metabolism and membrane remodelling. It is important to note that lipids are the predominant constituents

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(a)

| Stats | Value |
|---|---|
| Total_trinity_'unigenes' | 40508.00 |
| Total_trinity_transcripts | 65245.00 |
| Percent_GC | 38.86 |
| Contig_N10 | 5797.00 |
| Contig_N20 | 4313.00 |
| Contig_N30 | 3463.00 |
| Contig_N40 | 2825.00 |
| Contig_N50 | 2293.00 |
| Median_contig_length | 561.00 |
| Average_contig | 1163.70 |
| Total_assembled_bases | 75925282.00 |

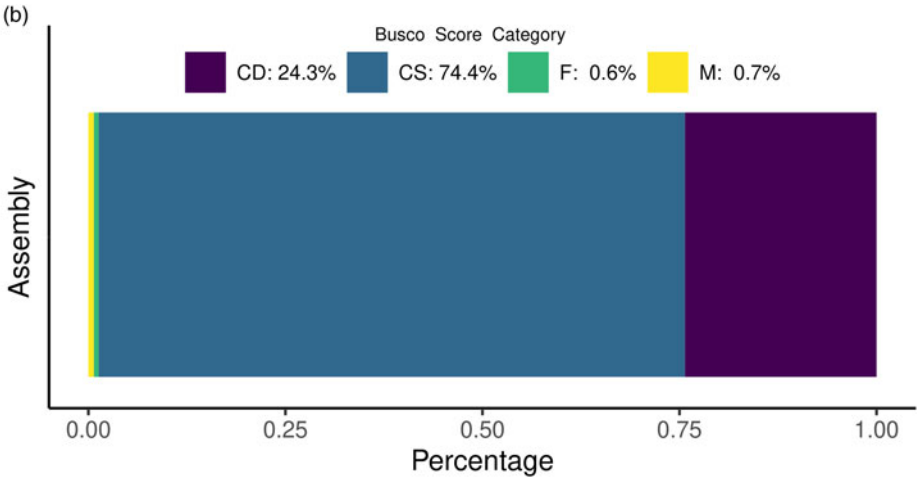


**Figure 1.** General transcriptome assembly statistics. (A) Metrics of the BUSCO program, (B) commonly used assembly descriptive statistics. CD, CS, F, and M correspond to the orthologue categories defined by BUSCO. CD, complete duplicated; CS, complete single; F, fragmented; M, missing.

of biological membranes and that membranes are the first targets of degradation during dehydration. Dehydration, in general, causes cytoplasmic crowding, which increases the likelihood of protein denaturation and membrane fusion (Hoekstra *et al.*, 2001). Therefore, the protection of membrane integrity is essential to maintaining metabolic homeostasis during an event of water restriction. It has previously been observed that alterations in phospholipid fatty acids in *D. melanogaster* are implicated in the differential response of lines showing rapid and slow recovery from chill coma, most likely due to the loss of cellular membrane homeoviscosity (Goto *et al.*, 2010).

Based on our findings, we suggest that cell membranes in flies exposed to desiccation conditions are likely to undergo membrane remodelling during these conditions. Thus, membrane remodelling could be an important process for the acclimatisation of flies to desiccation conditions, since the cell membranes of insects tend to be highly elastic with extremely low membrane tension (Shiomi *et al.*, 2021). Furthermore, there is evidence that membrane remodelling occurs across a wide range of organisms and is involved in the acquisition of desiccation tolerance (Gasulla *et al.*, 2013; Ren *et al.*, 2020). A similar scenario may occur in *A. ludens*. On the other hand, the activation of lipid metabolism may indicate a metabolic adjustment to increase lipid content, which in turn can be converted to metabolic water (Hadley, 1994; Chippindale *et al.*, 1998). Thus, these results could indicate that the induction of lipid metabolism is important because it provides a source of energy and water in this species and, as occurs in other insects, it may be useful

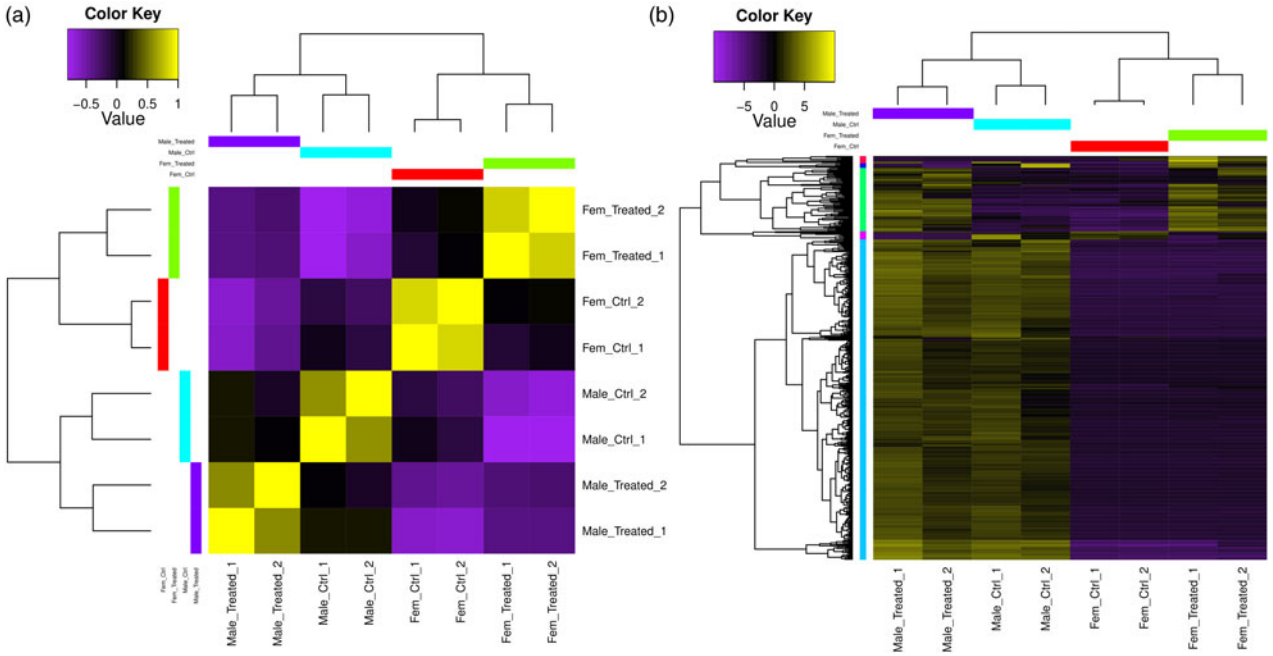


**Figure 2.** Differential expression pattern analysis. (A) Correlogram based on a Pearson correlation analysis of DEG abundances among libraries. (B) Heatmap clustering of the DEGs for each experimental group shows sample specificity.

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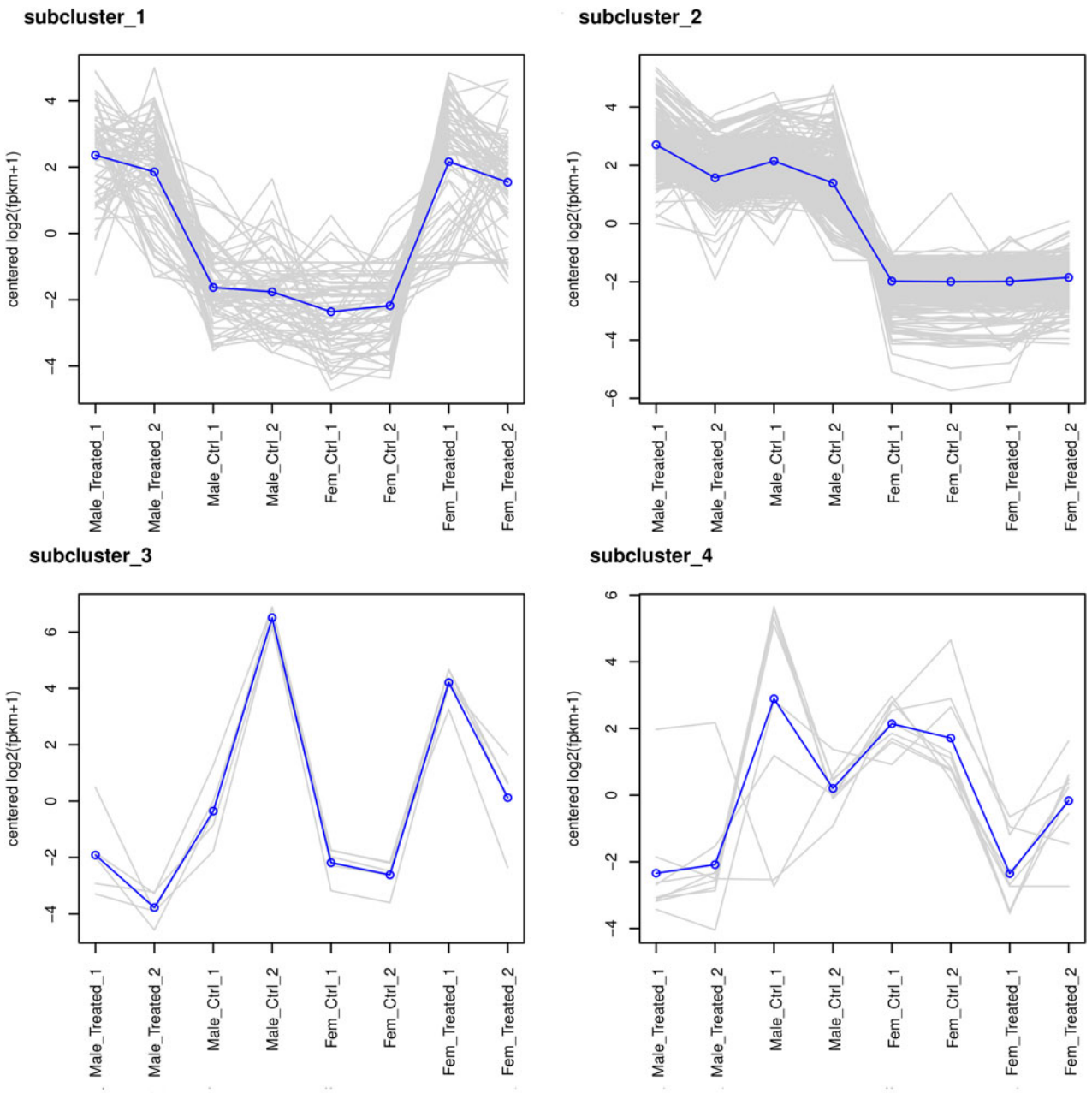


**Figure 3.** Mean expression profile of the gene clusters defined across samples.

when facing extreme conditions (Bong *et al.*, 2021). Therefore, we strongly encourage further lipidomic characterisation to compare the lipid profiles of fruit flies exposed and unexposed to desiccation.

Proteases play vital roles in processes like development, growth, metamorphosis, apoptosis, and immunity in insects (Saikhedkar *et al.*, 2015). Of particular interest is the induction of matrix metalloproteinase-2 (MMP2), a protein that belongs to a family of zinc-dependent proteases that are well-known for their ability to proteolyse extracellular matrix proteins throughout the body (Kandasamy *et al.*, 2010). MMP2 is also necessary and sufficient to induce fat-body remodelling (Bond *et al.*, 2011). It is worth noting that this idea is consistent with the induction of transcripts encoding lipid-related enzymes. Less evident is the putative role of the induction of digestive proteases, such as zinc carboxypeptidase A1 or trypsins, under the tested conditions, as the current data suggest that these genes are actively involved in digestion processes; in the case of trypsin, its regulation in fruit flies is usually activated upon ingestion (Brackney *et al.*, 2010; Li *et al.*, 2017a, 2017b).

The up-regulation of these transcripts raises the question of the possible role of the fat body in the response to desiccation in *A. ludens*. The fat body is an organ unique to insects that is relatively large and distributed throughout the insect body, preferentially beneath the integument and surrounding the gut and reproductive organs (Law and Wells, 1989), and many of the above-mentioned functions likely occur in or depend on this organ. In addition, most of the immune proteins present in the

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**Table 1.** Filtered list of the DEGs annotated in each cluster

| ID | Name | Cluster |
|---|---|---|
| XM_054883605.1 | Phosphatidate phosphatase LPIN3 (LOC129245438), transcript variant X6, mRNA | Cluster_1 |
| XM_054885813.1 | Protein SERAC1 (LOC129246998), transcript variant X1, mRNA | Cluster_1 |
| XM_054881845.1 | Matrix metalloproteinase-2-like (LOC129244231), mRNA | Cluster_1 |
| XM_054883758.1 | Solute carrier organic anion transporter family member 1A3-like (LOC129245548), transcript variant X1, mRNA | Cluster_1 |
| XM_054885449.1 | Rho guanine nucleotide exchange factor 7 (LOC129246818), transcript variant X3, mRNA | Cluster_1 |
| XM_054882846.1 | Trypsin beta-like (LOC129244931), mRNA | Cluster_1 |
| XM_054882316.1 | Trypsin alpha-like (LOC129244595), mRNA | Cluster_1 |
| XM_054883661.1 | Basic proline-rich protein-like (LOC129245481), mRNA | Cluster_1 |
| XM_054884510.1 | TATA-binding protein-associated factor 2N-like (LOC129246017), mRNA | Cluster_1 |
| XM_054883412.1 | Cyclic GMP-AMP synthase-like receptor (LOC129245328), mRNA | Cluster_1 |
| XM_054881741.1 | Basic salivary proline-rich protein 4-like (LOC129244125), mRNA | Cluster_1 |
| XM_054887342.1 | Zinc carboxypeptidase A 1-like (LOC129247959), mRNA | Cluster_1 |
| XM_054887548.1 | Zinc carboxypeptidase A 1-like (LOC129248112), mRNA | Cluster_1 |
| XM_054887976.1 | Seminal metalloprotease 1-like (LOC129248433), mRNA | Cluster_1 |
| XM_054885050.1 | Larval cuticle protein 4-like (LOC129246327), mRNA | Cluster_1 |
| XM_054887453.1 | Lysosomal aspartic protease-like (LOC129248041), mRNA | Cluster_1 |
| XM_054886120.1 | Pancreatic lipase-related protein 2-like (LOC129247156), mRNA | Cluster_1 |
| XM_054881967.1 | Transmembrane protease serine 9-like (LOC129244347), mRNA | Cluster_1 |
| XM_054884736.1 | Intermembrane lipid transfer protein Vps13 (LOC129246164), mRNA | Cluster_1 |
| XM_054882258.1 | Attacin-B-like (LOC129244562), mRNA | Cluster_3 |
| XM_054884352.1 | Diptericin A-like (LOC129245913), mRNA | Cluster_3 |
| XM_054883361.1 | Diptericin-D-like (LOC129245283), mRNA | Cluster_3 |
| XM_054886202.1 | C-type lectin 37Db-like (LOC129247194), mRNA | Cluster_5 |

haemolymph are synthesised in the fat body (Skowronek *et al.*, 2021). For example, we observed the induction of transcripts encoding for antimicrobial peptides (AMPs), such as attacin-B-like, diptericin A-like, or diptericin-D-like. In general, AMPs are cationic peptides with 15–100 amino acids that typically function at the membrane level by destabilizing prokaryotic membranes via non-specific electrostatic interactions (Melo *et al.*, 2009; Li *et al.*, 2017a, 2017b). The attacin gene family, for instance, regulates immune responses to protect *Hyalophora cecropia* (Lepidoptera) from bacterial infection (Hultmark *et al.*, 1983). The fat body is the organ responsible for the humoral response in *Drosophila* by synthesizing and secreting AMPs into the haemolymph (Zheng *et al.*, 2016; Won *et al.*, 2023). C-type lectins, on the other hand, are a family of carbohydrate-recognition domain-containing proteins associated with fat body function that play important roles in insect innate immune responses and gut microbiome homeostasis maintenance (Zhu *et al.*, 2020; Li *et al.*, 2021). However, it is highly intriguing that these immune-related components were up-regulated only in exposed females. This observation deserves a deeper, more detailed characterisation, but it could mean that the induction of the transcripts encoding for these AMPs is subjected to hormonal control.

Another interesting finding was the up-regulation of the larval cuticle protein 4-like. Structural cuticular proteins (CPs) are the primary component of insect cuticles and contribute to variations in the physical properties and functions of this structure. However, the exact role of CPs is not yet fully understood (Andersen *et al.*, 1995; Bazinet *et al.*, 2010). In the case of *A. ludens*, the up-regulation of the mRNA of this protein indicates that it plays a role in the response to desiccation.

Our results suggest that some responses in the up-regulation, especially those related to immunity, may be part of a common response to different stressors. Recent studies indicate that a common response to two or more stressors exists in different species, a phenomenon known as cross-talk and cross-tolerance, which enables insects to cope with changing environments successfully, allowing them to colonise new environments (Sinclair *et al.*, 2007; Bueno *et al.*, 2023). Further studies are needed to fully understand this strategy in fruit flies and other polyphagous insects.

In conclusion, our findings provide a framework for understanding the transcriptional response of *A. ludens* to desiccation conditions. These results can serve as a basis for the exploration of further questions arising from this study and lead to a better understanding of the molecular traits that may shape the evolution of this species in its future path through global warming and colonisation of new areas. Nevertheless, more studies with wild flies are necessary to further understand the observed molecular changes given that they differ from mass-reared flies in many physiological aspects, especially in the intensity of the response to field stressors since wild flies exhibit local adaptations to many environmental factors (Terblanche and Chown, 2007; Parvizi *et al.* 2024).

**Supplementary material.** The supplementary material for this article can be found at https://doi.org/10.1017/S0007485324000373.

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**Author contributions.** J. A. Z.-B.: formal analysis, reviewing, and writing; J. M. S.: writing. M. T. T., M. A. A.-V., and J. A.: experimental design and technical procedures; T. A.-I.: experimental design and reviewing; F. D.-F.: project manager, experimental design, conceptualisation, writing, and reviewing.

**Competing interests.** The authors state that no conflict of interest exists in this work.

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