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Offspring reverse transcriptome responses to maternal deprivation when reared with pathogens in an insect with facultative family life

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Offspring of species with facultative family life are able to live with and without parents (i.e. to adjust to extreme changes in their social environment). While these adjustments are well understood on a phenotypic level, their genetic underpinnings remain surprisingly understudied. Investigating gene expression changes in response to parental absence may elucidate the genetic constraints driving evolutionary transitions between solitary and family life. Here, we manipulated maternal presence to observe gene expression changes in the fat body of juvenile European earwigs, an insect with facultative family life. Because parents typically protect offspring against pathogens, expression changes were recorded in pathogen-free and pathogen-exposed environments. We found that manipulating maternal presence changed the expression of 154 genes, including several metabolism and growth-related genes, and that this change depended on pathogen presence. Specifically, localization and cell transporter genes were downregulated in maternal absence without pathogens but upregulated with pathogens. At least one immunity gene (pathogenesis-related protein 5) was affected by pathogen exposure regardless of maternal presence. Overall, our findings explicate how offspring adjust to parental deprivation on a molecular level and reveal that such adjustments heavily depend on pathogens in the environment. This emphasizes the central role of pathogens in family life evolution.

1. Introduction

Family life (i.e. the association of at least one parent with their offspring) is a taxonomically widespread form of social life in the animal kingdom [1–4]. From birds to mammals, insects and arachnids, these associations exhibit great variation in terms of duration and composition: they can last from a few hours to an entire lifetime, and encompass from one to multiple juveniles tended by either their mother, their father or both parents [5]. The presence of parents during family life is generally associated with the expression of care to juveniles, which ultimately enhances offspring development and survival until adulthood [5]. This can be achieved, for instance, when parents protect juveniles against predators and harsh climatic conditions or provide food resources [6,7]. In addition, parents often limit the risks of pathogen infection in their offspring by expressing the forms of social immunity [8,9], which can involve the transfer of antimicrobial agents to offspring via food (e.g. milk in mammals [10]), the grooming and cleaning of offspring to mechanically remove external pathogens [11] and/or nest

sanitation with the use of self-produced or collected materials with antimicrobial properties [9,12,13].

By alleviating or removing environmental hazards (such as pathogens), parents effectively create a micro-environment in which offspring can thrive. As a result, the loss of parents during family life (e.g. due to clutch desertion or early adult mortality) is expected to entail major changes in offspring life-history traits [14,15]. This has been shown, for instance, in the red deer *Cervus elaphus* [16], the killer whale *Orcinus orca* [17] and many birds [18], where the absence of tending parents overall increases the risk of natural death and/or compromises the physical conditions of offspring. This is also the case in both rats and monkeys, where parental deprivation hampers the mating success of adult offspring and diminishes the level of care these latter subsequently express towards their own descendants [14,19–22]. Finally, maternal deprivation can also alter offspring behaviours, such as in the Japanese quail where orphaned young are more fearful, more neophobic in novel environments, more aggressive, and less competent in executing spatial tasks than young previously reared with their mothers [23,24].

While the phenotypic responses of offspring to parental loss have been explored across numerous species and taxa [25–28], the molecular basis of family life has been mostly explored from the parental side. Over the last decade, several studies revealed that a number of genes expressed in parents are connected to their levels of investment into care, for example, in fishes [29] and insects [30–33]. By contrast, little is known on the offspring side [34,35], particularly when offspring do not rely on parental care to survive, and thus have the capability to live either with or without tending parents (i.e. in precocial, as opposed to altricial species)—a property that probably prevailed in the early evolution of social life [2]. Yet, shedding light on how these precocial offspring respond to the presence/absence of tending parents on a transcriptomic level would offer a unique opportunity to better understand the genetic and environmental constraints explaining how family life can evolve from a solitary state, why it can be maintained and/or disrupted over generations, and finally, how this may lead to highly integrated social systems exhibiting reproductive division of labour, such as eusociality [36–38].

Here, we investigated the impact of maternal loss on offspring gene expression in the European earwig *Forficula auricularia*. Because parents typically protect their juveniles against pathogens, the transcriptomic changes were recorded both in pathogen-free and pathogen-exposed environments. In the European earwig, mothers tend their offspring (called nymphs) after egg hatching for several weeks [39,40], during which they exhibit extensive forms of care such as allo-grooming, protection against nest intruders and food provisioning by regurgitation [7,39,41]. Mother–offspring interactions have long-term effects in earwig juveniles, as they shape the reproductive strategy of the resulting adult offspring [42], as well as the level of care the resulting adult offspring provide to their own descendants [43,44]. Nevertheless, offspring do not require post-hatching maternal care to develop and survive [36,44,45] since the gregarious, mobile nymphs are capable of foraging on their own [39] and can share food resources among their siblings via allo-coprophagy and proctodeal trophallaxis [26,46,47]. Previous studies have found that earwigs' resistance against pathogens may rely on group living and suggest that maternal loss may shape nymphs' ability to resist against pathogen presence in the nesting environment. Specifically, adults and juveniles collectively protect their nests against microbes by lining the walls with faeces exhibiting antimicrobial properties [12], and mothers increase their investment into egg care when their eggs or nesting area are covered with fungal spores [11,48]. Furthermore, early-life exposure to fungal spores induces increased offspring mortality during their first developmental instar as well as increased immune investment in adulthood [49]. Conversely, early maternal presence has no long-term effect on offspring investment into basal immunity [49]. Finally, the presence and nature of social contacts among earwig adults shape both their levels of basal immunity and their resistance against spores of the common entomopathogenic fungus *Metarhizium brunneum* [50,51].

In a full factorial experiment, we manipulated the presence of mothers in groups of nymphs maintained either with or without *M. brunneum* spores in the nesting environment and then tested the impact of maternal deprivation and pathogen exposure on gene expression in the abdominal fat body of these nymphs. The insect fat body is a multifunctional organ playing a central role in (i) lipid metabolism and storing/using energy reserves, two key factors in insect growth [52,53], and (ii) the production of antimicrobial peptides and proteins, two crucial parameters in insect immunity [54,55]. If earwig nymphs adjust to the social environment created by the mother, we expect the young nymphs to adjust their metabolism via gene expression changes to take full advantage of any parental resources. Conversely, if the absence of mothers causes stress for the nymphs, we would expect fat body gene expression to reflect reduced growth metabolism or increased immunity [50,56]. If earwig nymphs adjust their immune response to an environment featuring the presence (or absence) of pathogens in the nest, we predict an increased expression of immunity-related genes in nymphs indirectly exposed to the pathogen compared to the controls. Finally, if maternal presence facilitates nymphs' pathogen defence, as would be expected if maternal care is a form of social immunity [8,9,57], we predict that the presence of a mother buffers the effects of pathogens on nymph gene expression.

2. Materials and methods

(a) Experimental design

To investigate the effects of manipulating maternal presence in conjunction with or without pathogen exposure on gene expression in the earwig nymphs' fat body, we used 25 experimental clutches produced by *F. auricularia* females field sampled in July–August 2015 in Mainz, Germany (49°58′20.5″N 8°11′42.3″E). We reared these females under laboratory conditions until egg production and hatching using a standard protocol [49]. One day after egg hatching (all eggs typically hatch within 24 h; [39]), each experimental clutch was trimmed to 35 nymphs (mean original clutch size ± s.e. = 49.26 ± 2.1) and then transferred to recipient Petri dishes containing (i) their own mother and non-contaminated sand, (ii) their own mother and spore-contaminated sand, (iii) no mother and non-contaminated sand, or (iv) no mother and spore-contaminated sand. All clutches were then maintained under standard conditions (18–20°C, 12 : 12 h, dark : light) and provided with an *ad libitum* amount of standard food (detailed food composition in [26]), which was changed every 3 days until the nymphs were used for RNA extraction on day 10. The spore-contaminated and non-contaminated sands were obtained by preliminary grounding each recipient Petri dish (9 cm diameter) with humid sand and then sprinkling

the sand with 100 µl of either a conidiospore solution of *M. brunneum* diluted in 0.05% Tween 80 (10^7 spores per ml), or a spore-free 0.05% Tween 80 solution, respectively. Note that *M. brunneum* is a natural pathogen of *F. auricularia* and is known to be infectious and lethal to a wide range of insect species, including the European earwig [48–50].

(b) RNA extraction and sequencing

Ten days after we set up the clutches in one of the four experimental treatments, we haphazardly selected one nymph per clutch to extract its fat body and conduct a standardized RNA extraction. At this time, nymphs are still in their first developmental instar (the stage during which family life mainly occurs [36]) but exhibit greater mobility and independence than immediately after hatching (M.K. 2020, personal observation). Each nymph was first removed from its experimental clutch and instantly killed by decapitation; its abdominal fat body was immediately extracted on ice and homogenized in TRIzol (Invitrogen, ThermoFisher Scientific, Waltham MA, USA) and stored at −20°C. The extraction process was conducted using the RNAeasy mini extraction kit (Qiagen) following the corresponding protocol. RNA samples were stored at −80°C after extraction. Library preparation (Truseq Transcriptom Library Construction) and sequencing of 100 bp paired end reads on an Illumina HiSeq 2000/2500 was conducted at BGI-Hongkong. Out of the 28 total samples submitted for sequencing (7 per treatment), three were excluded due to low RNA content, resulting in a final sample size of $n = 7$ for own mother and sand, $n = 6$ for own mother and spore-contaminated sand, $n = 5$ for no mother and non-contaminated sand and $n = 7$ for mother and spore-contaminated sand (total $n = 25$).

(c) Sample quality check and transcriptome assembly

The quality of the raw reads was assessed using *FastQC* v. 0.11.5 (Babraham Bioinformatics) in conjunction with *MultiQC* v. 1.2 [58] followed by removal of Illumina adapter sequences using *Trimmomatic* v. 0.36 [59]. After testing several de novo assembly approaches, we compared results using *Transrate* v. 1.03 [60] and went forward using the results obtained by *CLC Assembly Cell* based on the number of contigs, backmapping rate and number of *n*'s (details of all approaches see electronic supplementary material, table S1).

(d) Gene expression analysis

For the gene expression analysis, reads were aligned to the assembly contigs and read count abundances were obtained using *Kallisto* v. 0.43.1 (electronic supplementary material, table S2) [61] and corrected for library size using the package *tximport* [62]. To test whether mother and/or pathogens presence triggered any changes in gene expression in the nymph fat body, we used the likelihood ratio (LRT) test of the R package *Deseq2* v. 1.16.1 [63] to compare the goodness of fit of several models. First, we obtained a list of differentially expressed genes (DEGs) affected by only maternal presence/absence by comparing a full model fitted with both treatment factors (mother presence + pathogen exposure) against a reduced model fitted only with pathogen presence. Second, we tested for DEGs only affected by pathogen exposure by comparing the same full model (mother presence + pathogen exposure) against a reduced model fitted with maternal presence. Finally, to detect DEGs that were affected by an interaction of the two factors, we compared a full model fitted with an interaction of the terms (mother presence × pathogen exposure) against a reduced model fitted with the main effects but no interaction (mother presence + pathogen exposure). These LRT comparisons were used for significance testing of any DEGs, and the resulting *p*-values were adjusted for multiple comparisons using the Benjamini & Hochberg correction [64] implemented in *DESeq2*. Genes with significant interaction were clustered according to their expression patterns (see below) and the scaled gene counts (Z-score) of each cluster were subjected to *t*-tests to determine specific treatment differences in gene expression. The resulting *p*-values were adjusted for multiple comparisons using the Bonferroni method. Genes were considered significantly differentially expressed if adjusted $p \leq 0.05$. Visualization of all interaction DEGs via heatmap was done using the R package *pheatmap* v. 1.0.12 [65] using the scaled expression option (displaying Z-score).

(e) Identifying expression patterns

To visualize the patterns of gene expression within the DEGs and to detect common expression patterns across treatments of genes of interest, we used the R package *DEGreports* v. 1.18.1 to calculate and create plots of clusters of differentially expressed contigs with similar expression [66]. This was done by obtaining normalized count data with the *rlog* function of *DESeq2* and running *DEGreports* on the contigs represented in our DEG using default settings (min. cluster size = 5).

(f) Annotation and enrichment analyses

We submitted all contigs to a local *BlastX* (NCBI) search against the non-redundant invertebrate protein database (state June 2017) for possible annotations. Furthermore, we employed gene enrichment analysis to identify overrepresented functional groups in each DEG list. To this end, we first used *Transdecoder* v. 5.0.2 [67] to translate our de novo assembled contig nucleotide sequences into amino acid sequences and then used these to conduct an *Interproscan* v. 5.26–65 [68] search. Enrichment analysis of gene ontology (GO) terms was performed using the R package *topGO* v. 2.28.0 using the *parentchild* algorithm [69]. The list of genes of interest was comprised differentially expressed genes with similar expression patterns (differential expression defined by *DEseq2*, expression patterns by *DEGreports*). The *p*-values for each GO term were obtained by using an exact Fisher test and an FDR correction for multiple testing.

3. Results

(a) Gene expression analysis

Our LRT model comparisons revealed that no genes were solely affected by maternal presence/absence, whereas our manipulation of pathogen exposure overall resulted in 12 differentially expressed genes (DEGs). Among these 12 genes, 5 received an annotation from the BlastX search, including one immunity-related gene (*Pathogenesis-related protein 5*, FDR $p = 0.008$; table 1). These pathogen-associated DEGs were all upregulated in the presence of the pathogen (table 1; electronic supplementary material, figure S1).

In addition to these main effects, the analysis comparing models with and without interaction term showed that our manipulation of maternal and pathogen presence interacted to shape the expression of a total of 154 genes (figure 1; electronic supplementary material, table S3). Among these, 108 received a BLAST annotation. Interestingly, 150 of these 154 DEGs can be grouped into two distinct expression clusters consisting of 29 (cluster 1) and 121 genes (cluster 2) (figure 2). In cluster 1, maternal absence upregulated gene expression when there was no pathogen (*t*-tests comparing Z-scores in figure 2; $t = -40.17$, d.f. = 48.71, Bonferroni-adjusted $p < 0.001$), but downregulated it in presence of pathogens ($t = 13.14$, d.f. = 55.96, adjusted $p < 0.001$). By contrast, maternal

Table 1. Annotated differentially expressed genes (DEGs) affected only by pathogen exposure (no interaction with maternal presence/absence). Positive log-fold-change indicates the upregulation of expression in the presence of the pathogen. Overall, 12 genes were affected by pathogen exposure alone (7 unannotated). For a visualization of the expression patterns, see electronic supplementary material, figure S1.

gene annotation	species (annotated)	log_2-fold change	FDR *p*-value
pathogenesis-related protein 5	*Tribolium castaneum*	4.795264	0.008
serine/threonine-protein phosphatase 6 regulatory ankyrin repeat subunit b-like	*Strongylocentrotus purpuratus*	3.662594	0.046
AAEL007486-PA	*Aedes aegypti*	3.095392	0.024
protein ZBED8-like	*Acyrthosiphon pisum*	3.693886	0.021
uncharacterized protein LOC105664042 isoform X2	*Megachile rotundata*	9.152959	0.001

absence downregulated the expression of genes present in cluster 2 in the absence of pathogens ($t = -83.61$, d.f. = 225.66, adjusted $p < 0.001$), whereas it did the opposite when pathogens were present ($t = -25.89$, d.f. = 205.56, adjusted $p < 0.001$). Note that none of the 12 DEGS solely shaped by pathogen exposure were similar to or are included in the 154 DEGs shaped by an interaction between maternal and pathogen presence. For a full overview of BLAST annotations and *p*-values, see electronic supplementary material, tables S2 and S3.

(b) Gene enrichment analyses

From the 12 DEGs associated with pathogen exposure, the only significantly enriched biological process was *multi-organism process* (table 2). Associating the two clusters of similar expressed contigs among the DEGs affected by the interaction of maternal and pathogen presence revealed enrichment of several growth and metabolism-related functions (table 2). Specifically, the genes from cluster 1 produced a GO term associated with growth and its regulation, while genes from cluster 2 were associated with a number of different functions mostly related to cell transport and metabolism processes (table 2).

4. Discussion

Offspring living in facultative family systems can receive short- and long-term benefits from parental care but still survive in the absence of a caring parent [14,44]. In this ancestral form of social life, we expect offspring to adjust to key attributes of their rearing environment, such as parental care and/or pathogen presence, for instance by altering their behaviour, development or energy metabolism [26,51,70]. However, very little is known about the molecular basis underlying such offspring adjustments. In our study, we addressed this lack of information by exploring the gene expression differences between earwig nymphs reared either with or without their mother in conjunction with the presence and absence of pathogens in the nesting environment. In line with our predictions, we found that maternal deprivation is associated with gene expression changes in the nymphs' fat body, and that these changes appear to include several metabolism-related genes. However, we found that these adjustments depend on pathogen exposure. In particular, a cluster of genes partially associated with GO terms related to growth and its regulation (cluster 1) was upregulated in maternal absence when pathogens were absent, whereas it was downregulated when pathogens were present. Conversely, a cluster of genes including genes associated with GO terms related to cell transport and metabolism processes (cluster 2) was downregulated in maternal absence when pathogens were absent, whereas it was upregulated when pathogens were present.

The reported effects of maternal absence on offspring gene expression in the absence of pathogens offer an important first glance at the molecular underpinnings governing the unique ability of juveniles from facultative species to survive and thrive without maternal care (see also [34,35]). Past studies and theory strongly suggest that these offspring should dynamically attempt to maximize the benefits from care and/or seek rapid dispersal and development to minimize costs in its absence [5,70,71]. Our data are in line with these predictions. They hint at involvement of specifically metabolism-related genes, which are probable centrepiece for adjustments to already-known differences in begging, food acquisition and sharing behaviours in earwig juveniles receiving or not receiving maternal care [26,46]. The explorative nature of our study, however, limits the possibility for an interpretation of the specific effects of the differentially expressed genes (i.e. on specific phenotypes), especially in the light of incomplete annotations and functional analyses. Our work therefore calls for further studies investigating the specific duration, role and mechanism of the molecular changes presented here. For instance, the role of metabolism-related genes in the adjustment to the presence of care may be revealed by experimental manipulations of food availability and the resulting quantification of food provisioning and sharing behaviours between family members.

Somewhat surprisingly, we found that the expression of most genes related to maternal absence was reversed in the presence of the pathogen. The phenotypic impact of early pathogen exposure on earwig offspring has been shown (using an identical set-up) to include a reduced survival during development and an increased innate immunity (i.e. haemocyte concentration) in adulthood, but these impacts were overall independent of the maternal presence/absence [49]. Conversely, the present data suggests that the presence of a potentially lethal pathogen in the rearing environment may completely change molecular adjustments to the absence of a caring mother. This interaction either (i) reflects dynamic alterations of nymphs' responses shaped by both maternal and pathogen presence or (ii) is caused by simultaneous and conflicting effects on fat body pathways directly or indirectly associated with both growth metabolism and immune activation [52]. The first scenario could occur if the presence or

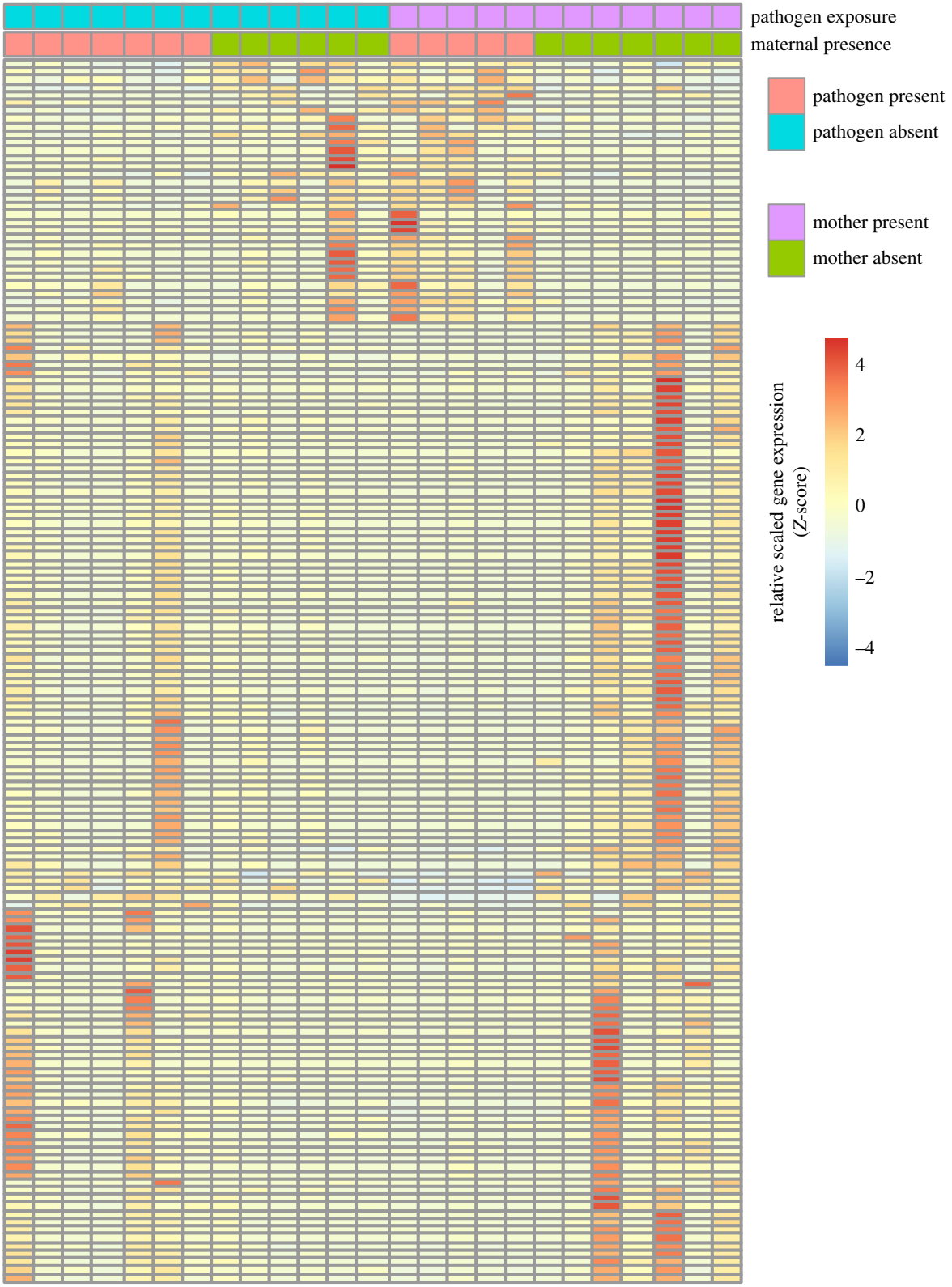

Figure 1. Heatmap of all 154 differentially expressed genes (DEGs) depending on the interaction of the pathogen exposure and maternal presence treatments. Columns represent individual samples ($n = 25$) ordered by treatment. Rows depict the degree of gene expression normalized across samples per row (Z-score). For a full list of all 154 DEGs with available annotations, see electronic supplementary material, table S3. Note that no gene's expression was affected by maternal presence alone (i.e. outside of this interaction), while a total of 12 genes were differentially expressed depending on pathogen exposure alone. Since none of these genes overlap with the 154 DEGs from the interaction, they are not depicted here. (Online version in colour.)

absence of a caring mother actively shapes offspring responses to a pathogenic environment. Parental care is well known to affect offspring immunity directly through care behaviours, such as external and social immunity [9,12,27,72]. Even though previous investigation in this species showed no effects of an interaction between pathogen and maternal presence on long-term immunity or survival [49], short-term adjustments cannot be ruled out here. Conversely, offspring exposure to pathogenic environments may alter how mothers invest into care: earwig maternal care depends on the offspring quality chemically perceived by the mothers [73] and nymphs' social behaviours are shaped and informed by their mothers'

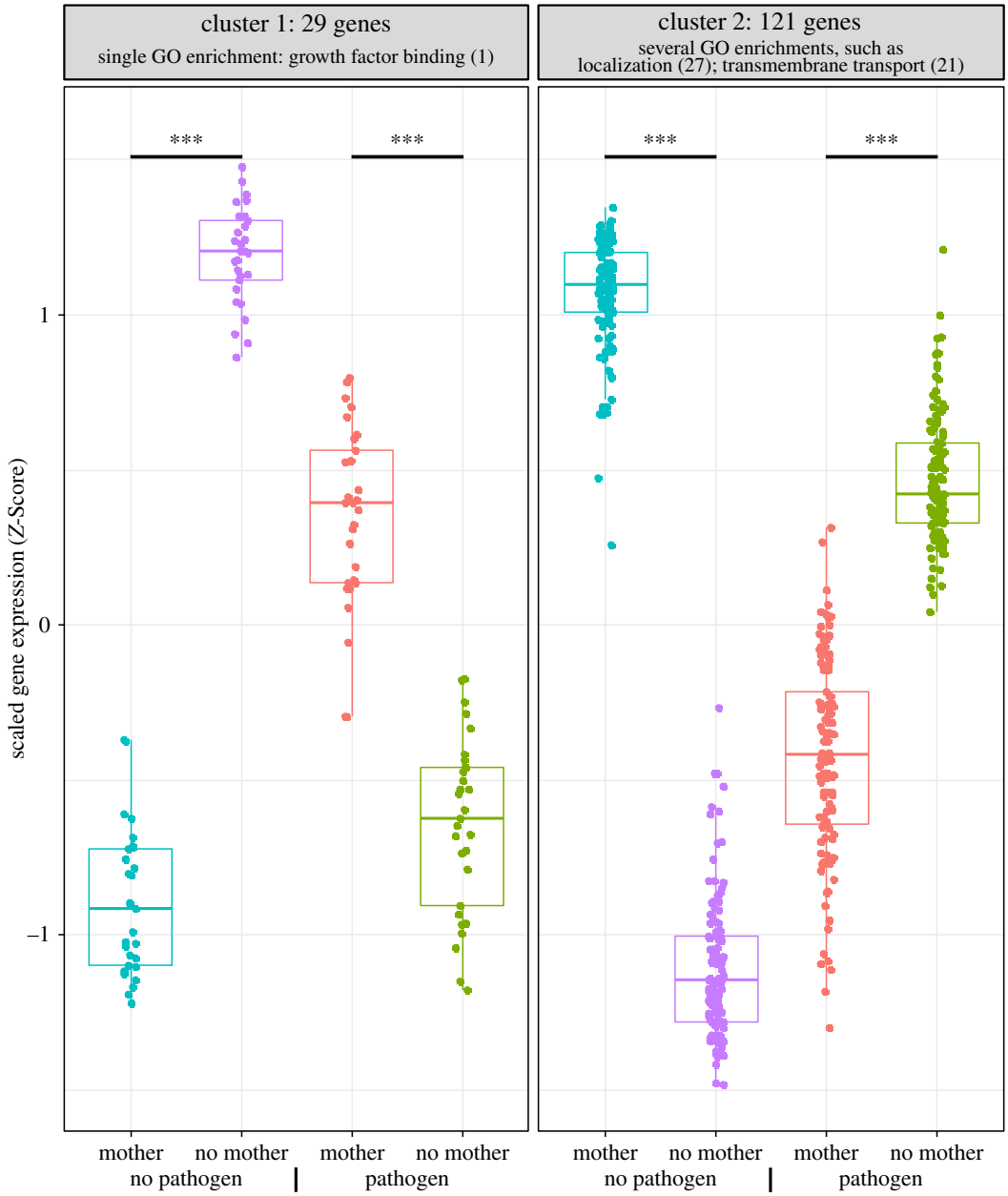

Figure 2. Clustering and visualization of 150 (out of a total 154) differentially expressed genes that were clustered using similar expression patterns, based on the interaction of maternal and pathogen presence/absence. These genes grouped in the two clusters visualized above (29 and 121 genes). While only a single functional category (represented by a single gene only) was enriched in Cluster 1, genes in Cluster 2 were enriched for metabolic localization and transport (table 2). Asterisks denote statistical differences (all $p < 0.001$, *t*-tests, details in Results section). Expression pattern analysis and subsequent clustering was done using degreports. Degree of gene expression has been normalized across genes (Z-score). For a full list of genes in each cluster, see electronic supplementary material, table S3. (Online version in colour.)

condition [74,75]. If pathogen exposure affects the chemical cues governing these mechanisms, pathogenic environments would fundamentally alter social interactions, and thus their associated benefits from family members. Note, however, that we do not detect the differential expression of CHC synthesis-associated genes (such as elongases and desaturases [76–78]). Deciphering which aspects of family interactions and/or energy availability may govern the observed gene expression changes would require cross-manipulation of the quality of care (or caring mothers), chemical communication and pathogen exposure in experimental families.

A second scenario to explain why the pathogen presence completely changes offspring molecular adjustments to maternal absence is that the combined manipulation of these two factors affects the same or connected gene pathways in the fat body (e.g. governing energy allocation in general). If this holds true, the observed interaction would merely reflect a partial masking or amplification of effects instead of indicating purposeful adjustments to the presence or absence of care. Mounting an immune response is typically associated with energetic and metabolic costs [79–81] so that the shared importance of the fat body in both immunity and metabolic regulation [52,55] may lead to important trade-offs in this tissue [82,83]. This overall emphasizes that joint adjustments to multiple environmental factors may result in unique expression patterns in the fat body [84]. It is important to note that the two scenarios might affect several genes included in the observed interactions simultaneously and are not necessarily mutually exclusive.

Finally, we showed that not all pathogen-associated gene expression changes were tied to manipulation of maternal presence. We observed one annotated immunity-related gene that

Table 2. List of significantly enriched GO functions associated with the differentially expressed genes along with the number of genes associated with the term in the reference transcriptome (reference) and in the respective DEG lists (significant). GO terms were obtained using topGO with the *parentchild* setting. Note that some significant terms are only represented by a single contig, which may affect any interpretation drawn from them.

DEG List	GO.ID	term	reference	significant	*p*-value
pathogen exposure	GO:0051704	*multi-organism process*	9	2	<0.001
maternal presence × pathogen exposure *Cluster 1*	GO:0019838	*growth factor binding*	2	1	0.006
maternal presence × pathogen exposure *Cluster 2*	GO:0051179	*localization*	730	27	<0.001
	GO:0055085	*transmembrane transport*	354	21	0.002
	GO:0072348	*sulfur compound transport*	8	3	0.001
	GO:0006820	*anion transport*	39	4	0.0038
	GO:0043647	*inositol phosphate metabolic process*	3	1	0.007
	GO:0006022	*aminoglycan metabolic process*	64	2	0.01
	GO:1901616	*organic hydroxy compound catabolic process*	3	1	0.014
	GO:0006508	*proteolysis*	402	4	0.014
	GO:0046834	*lipid phosphorylation*	6	1	0.02
	GO:0035434	*copper ion transmembrane transport*	1	1	0.023
	GO:0000103	*sulfate assimilation*	1	1	0.026
	GO:0015748	*organophosphate ester transport*	5	1	0.032
	GO:0055114	*oxidation-reduction process*	445	7	0.036
	GO:0046838	*phosphorylated carbohydrate dephosphorylase*	2	1	0.035
	GO:0017144	*drug metabolic process*	141	2	0.04
	GO:0000041	*transition metal ion transport*	3	1	0.043
	GO:0044262	*cellular carbohydrate metabolic process*	18	1	0.047

appears unaffected by maternal presence: *pathogenesis-related protein 5* (*prp5*). Pathogenesis-related proteins represent a conserved group of proteins with antimicrobial and antifungal properties such as osmotin and thaumatin and are involved in insect immune defence, for example, in the flour beetle *Tribolium castaneum* [32,85,86]. The pathogen-dependent expression of this gene regardless of maternal presence indicates that at least some immunity pathways in the fat body appear to be independent of any hypothesized energy adjustments in response to maternal care. Interestingly, this upregulation is not reflected in available phenotypic data where phenoloxidase, prophenoloxidase and haemocyte counts were measured from earwig offspring at the same developmental stage (10 days after hatching) under identical laboratory and treatment conditions [49]. This suggests *prp5* may not be tied to immediate expression of these key elements insect immunity [87] and instead involved in detecting an infection [88]. Whether an overexpression of immune-related genes such as PRP5 during early development is associated with higher immunity measurements, as suggested by [49], remains, however, unknown. In addition, only 5 out of the 12 genes exclusively affected by the pathogen presence received an annotation. Further studies are thus required to elucidate which and to what degree immune responses occur independently of the social environment, and how they shape immediate and future immunocompetence.

Our study focuses on gene expression changes in the insect fat body, an organ of significant biosynthetic and metabolic activity [89]. In addition to its role in insect immunity [55,84,90], most of what is known about insect energy expenditure and storage occurs in the fat body and is known to dynamically adjust to the physiological needs during development [52,53]. As such, the presence or absence of a caring mother is expected to have profound impact on the activity in the fat body, for instance, in terms of storage and mobilization of proteins and amino acids relevant for morphogenesis [52]. Our data confirm these expectations, but show that the degree and direction of the majority of the adjustments apparently associated with maternal presence strongly depend on the presence of pathogens, which represent a costly environmental hazard to animals—particularly in social contexts due to the increased infection risk associated with group living [8,9,57,91]. While a concrete identification of the observed effect based on our data is elusive, the distinct role of the insect fat body in both metabolism and immune system suggests several, non-mutually exclusive processes causing this interaction of maternal and pathogen presence on gene expression. Ultimately, our results emphasize the need for future works exploring the behavioural implications of maternal deprivation in nymphs. To this end, it is necessary to explore gene expression changes in other tissues, such as the brain, throughout offspring ontogeny, and possibly delve into the sensory inputs that drive

offspring adjustments, such as chemical communication between offspring and the caring parent, or the identification of pathogenic agents in the environment.

5. Conclusion

Our study overall provides the first insights on how maternal care and pathogens interact to shape offspring gene expression in a species with facultative family life. In particular, we revealed that variation in maternal presence affects gene expression in the fat body, including genes that are associated with metabolism and growth, and importantly, that these gene expression changes greatly depend on pathogen exposure. This indicates that pathogenic environments may dictate how offspring adjust their energy allocation to maternal presence, and thus stresses the central importance of environmental conditions, particularly pathogens, in the evolution of parental care. Our results indicate a complete reversal of offspring adjustments to maternal care when exposed to the pathogen, which may represent the first transcriptome evidence for an offspring strategy to adjust crucial life-history properties such as growth speed or dispersal from the nest to either avoid prolonged exposure to pathogens or to receive more resources through prolonged parental care. Overall, this study sheds light on the molecular basis of offspring response to changes in parental presence and suggests that pathogens probably shaped the use and function of parental care during family evolution.

Data accessibility. Raw RNA sequences and the de novo assembly are available on the NCBI Sequence Read Archive (Project ID: PRJNA477302).

Authors' contributions. J.M., B.F. and M.K. designed the experiment. M.K. and F.V. carried out the implementation. M.K. analysed the data with input from S.F., B.F., R.L. and J.M. All authors discussed the results and contributed to the final manuscript.

Competing interests. We declare we have no competing interests.

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