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Original Article

Parasite prevalence in a social host has colony-wide impacts on transcriptional activity and survival

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Abstract

Parasites pose significant challenges not only to individual hosts but also to entire social groups. We investigated the effects of parasitism by the cestode *Anomotaenia brevis* on colonies of its intermediate host, the ant *Temnothorax nylanderi*. We evaluated changes in worker and queen survival rates and transcriptional activity in the fat body of infected and uninfected workers, as well as in the parasite itself, in relation to infected worker prevalence and colony size. Cestode-infected workers are known to exhibit a significantly extended lifespan compared with uninfected workers. Here, we demonstrate that the survival rates of infected workers, uninfected queens, and uninfected workers decrease with increasing infected worker prevalence and increase with colony size. Transcriptomic analysis revealed stress-related signatures in all workers, regardless of infection status, as infection prevalence increased. Moreover, gene expression patterns, particularly in uninfected workers, were strongly influenced by colony size. The transcriptional activity of the parasitic cestode also shifted with infected worker prevalence, highlighting the complex dynamics of host–parasite interactions. These results demonstrate that parasites in social species impose colony-wide impacts that extend beyond infected individuals, even in the absence of direct cross-nestmate infection risks. Moreover, the consequences of parasitism can be modulated by colony size.

Keywords: parasite prevalence, parasite ecology, cestode, social insect, transcriptomics

Introduction

The disappearance or introduction of a species into an ecosystem can have a profound impact on community structure and dynamics (Crowl et al., 2008; Zavaleta et al., 2009). Parasites, in particular, are important drivers of biodiversity and evolution (Hudson et al., 2006; Mouritsen & Poulin, 2002): Their presence can influence the dynamics of host populations (Sinclair, 1979; Thomas & Bonsall, 2005), alter competition between species (Price et al., 1986), and shift ecosystem energy flows (Odum & Barrett, 1971).

An intriguing aspect of parasitism is that its influence can extend beyond direct hosts, potentially impacting social groups and ecological communities. In the bird *Phalacrocorax aristotelis*, nematode parasitism affects all family members, even those not directly infected (Granroth-Wilding et al., 2015) and in the fish *Oryzias latipe* mere exposure to a brain parasite alters behavior, even if infection does not occur (Vindas et al., 2023). We expect indirect effects of parasitism to be particularly important in social animals, which are often the target of parasites and pathogens (Hart & Hart, 2018), as frequent close contacts increase the transmission and spread of pathogens (Romano et al., 2022). Indeed, the risk of infection can increase with group size and relatedness (Webber et al., 2017). In particular, social insects are strongly affected by pathogens in their colonies. In honeybees, e.g., the presence of the microsporidian *Nosema* or viruses not only increases the tendency of infected bees to drift into foreign colonies (Fries & Camazine, 2001), but also the acceptance of drifters by uninfected bees (Forfert et al., 2015). Due to the strong selective pressure from parasites, social insects have evolved complex antiparasitic behaviors collectively termed as social immunity (Cremer et al., 2007, 2018), which includes changes in the interaction network structure (Stroeymeyt et al., 2014), adaptive grooming behavior (Stock et al., 2023), or prophylactic distribution of antipathogenic substances (Chapuisat et al., 2007). In addition, collective behaviors such as temperature regulation within honeybee colonies are affected by parasites, as shown by the link between temperature fluctuations and disease prevalence in the UK (Rowland et al., 2021) and reduced winter survival of infected colonies (Minaud et al., 2024). However, the consequences of nestmate infection and social immunity behaviors on uninfected nestmates remain understudied.

The host–parasite system of the tapeworm *Anomotaenia brevis* and its intermediate host, the ant *Temnothorax nylanderi*, is an excellent model to investigate parasite-induced changes in uninfected nestmates. Several studies have shown that *T. nylanderi* workers living in colonies with ants infected by cysticercoid cestode larvae exhibit

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altered behavior, changes in brain transcriptional activity, and reduced survival rates compared with workers in colonies without infected nestmates (Beros et al., 2015, 2021; Feldmeyer et al., 2016). These changes could be driven by the pronounced phenotypic shifts, particularly in behavior, of cestode-infected workers, who are unable to directly transmit the infection to their healthy nestmates. Infected workers are smaller, rarely take over work, and are mostly inactive. They have a less sclerotized and pigmented cuticle (Plateaux, 1972) and exhibit muscle atrophy (Feldmeyer et al., 2016). Intriguingly, they exhibit a severalfold extended lifespan (Beros et al., 2021), which could, in addition to direct manipulation by the cestode, result from uninfected nestmates providing increased care to infected colony members. The infection of some workers with *A. brevis* has pronounced colony-level consequences as well. Infected colonies produce a male-biased sex ratio and are also more likely to raise intercastes that are morphologically intermediate between workers and queens, both of which are known indicators of colony-level stress (Scharf et al., 2012).

T. nylanderi ants become infected when they ingest cestode eggs as larvae. The tapeworms then develop into cysticercoid larvae, which reside in the ants' hemolymph until the final host, woodpecker, preys on the infected workers (Plateaux, 1972). *A. brevis* cysticercoids do not remain dormant. They instead remain physiologically highly active and secrete proteins into their intermediate host: Of all proteins in the hemolymph of infected workers, 7% originate from the tapeworm (Hartke et al., 2023). Transcriptional changes in various tissues of infected host workers, including the brain, gaster, and fat body, result from altered larval development as well as shifts induced by active secretion from the parasite in adult hosts (Feldmeyer et al., 2016; Sistermans et al., 2023; Stoldt et al., 2021). The latter likely depends on parasite load, which can range from a single cestode infecting an individual ant to as many as 74 cysticercoids (Sistermans et al., 2023). Transcriptional shifts do not only occur in infected workers, but also in uninfected ants from infected colonies (Feldmeyer et al., 2016; Sistermans et al., 2023), which can be explained by the additional workload imposed on them due to the idleness of infected workers (Shik, 2010). As the workload of healthy workers increases with the prevalence of infected workers in the colony, this could impact the survival rates of healthy nestmates, including the queen, as well as their molecular physiology. The prevalence of infected workers varies significantly between colonies, ranging from a single infected individual to 70% of the workers. Additionally, larger colonies may better mitigate the effects of parasite prevalence by buffering the increased workload.

We aim to use this variation in infected worker prevalence and identify its colony-level consequences on the transcriptional activity of infected and uninfected workers, as well as on the parasite itself. We complement our novel transcriptomic analyses with a reanalysis of a multiyear survival dataset of the same population (Beros et al., 2021) to understand the effects of infection prevalence on the colony demography. While infection is known to impact colony-level traits including those of uninfected workers, previous work pooled all infected colonies into one category and thus did not consider between-colony variation in the prevalence of infected workers. A higher prevalence likely results in increased competition for care and food among infected workers and the queen with potential effects on the parasite. We thus examine whether colony-level prevalence impacts the expression of parasite traits as well. We expect parasites in colonies with higher parasite prevalence to exert a stronger influence on specific host traits such as stress, metabolism, physiology, and behavior. For both the transcriptomic as well as the demographic analysis, we also investigated the effect of colony size, as a larger workforce may better handle the stress of numerous infected individuals.

Methods

Sampling, experimental design, and sequencing for the transcriptome analyses

We collected 153 *T. nylanderi* colonies in dead maple wood between November 2019 and February 2020 in the Lenneberg Forest in Mainz, Germany (50.011605″N 8.174941″E), of which 33 colonies contained cestode-infected workers (rate of infected colonies was 22%). The rate of infected colonies at this site has therefore diminished somewhat from an earlier reported 30% in 2012 (Scharf et al., 2012). The ant colonies were transferred to artificial nest sites, consisting of a Plexiglas perimeter with a lid and base made of a microscope slide. The nest sites were covered with red foil and placed into a three-chambered nest box with a plaster floor. Each colony was fed twice weekly with crickets and honey and provided with water ad-libitum. We counted the number of queens, healthy workers, and infected workers and selected 16 infected colonies with a prevalence of infected workers between 2% and 72%. We deliberately did not include healthy colonies in our analyses because the influence of colony infection status on transcriptional activity has already been thoroughly investigated elsewhere (Feldmeyer et al., 2016; Sistermans et al., 2023; Stoldt et al., 2021). To be included in the samples, we also required that the colony had a queen, since her absence strongly influences the transcriptional activity in *Temnothorax* workers (Negroni et al., 2021).

Note that for the transcriptomic study, we did not include healthy colonies, as the influence of colony infection status in itself on transcriptional activity is already known (Feldmeyer et al., 2016; Sistermans et al., 2023; Stoldt et al., 2021). The selected colonies varied in size (16–177 workers; mean 77 workers), which gives us the opportunity to examine a potential interaction between infected worker prevalence and colony size.

The exact ages of the colonies are not known (queens can live for up to 20 years; Plateaux, 1986), and we cannot exclude a positive correlation between colony size and age. Importantly, however, they were likely all mature, since they no longer contained any workers of the first generation, which can be recognized by their small size, and 10 workers are sufficient for a colony to produce sexuals (Plateaux, 1972).

Infected workers are recognizable by their yellow cuticle, though a small (<10%) percentage of workers in infected colonies may display the healthy brown phenotype despite being infected (Scharf et al., 2012). Correcting "false negatives" with respect to infection status requires lethal dissection. We did this for healthy workers that were included in the transcriptome analysis (to ensure they were uninfected), while we considered cuticle coloration a sufficiently

accurate proxy for assessing infection prevalence in the colony as a whole.

All infected workers and a similar number of uninfected workers were dissected in the summer of 2020. As our primary goal was to compare the transcriptional activity in the fat body of these two standardized worker groups in relation to prevalence and colony size, we did not perform transcriptomics of the queen or foragers (while earlier survival data existed for these groups too, including uninfected colonies; we fully capitalized on data availability in that context, see the "Survival analysis" section below). The uninfected workers were selected at random apart from the sampling location, for which we chose the position in the nest near the brood, where the infected workers reside (Scharf et al., 2012). This made the sample focus on young brood carers, metabolically closest to the infected ants (Beros et al., 2021).

We isolated the fat body from each worker by removing the gut and trachea from the first gaster segment, leaving only the fat cells, which were placed in a vial containing 50 µl Trizol. In infected ants, we also removed the cestodes, located in the hemolymph around the midgut, and placed them in a separate vial with 50 µl Trizol. Samples were frozen on dry ice and subsequently transferred to −80 °C. To control the parasite load, which strongly influences the gene expression of infected workers (Sistermans et al., 2023), we selected one infected worker per colony whose cestode number was closest to the median of 7 (min = 5, max = 13), we did not encounter an infected worker with a number of cestodes within this interval in one colony (colony B; Supplementary Table S1). So, for this colony, we only have an uninfected worker. For each colony, we included all cestodes from this worker and the fat body of an uninfected nestmate. RNA was extracted using the Qiagen RNeasy extraction kit, RNA extraction failed for a single sample from an uninfected colony (colony F; Supplementary Table S1). We sent the rest of the samples to Novogene (Cambridge, UK) for messenger RNA library preparation using a NEBNext Ultra RNA Library Prep Kit. For cestode samples, a SMARTer amplification step was performed to accommodate low RNA content. The libraries were sequenced on an Illumina NovaSeq 6000 in paired-end 150-bp mode, yielding a minimum of 9 Gb per sample. RNA sequencing (RNA-seq) data for four of the uninfected workers were also utilized in Sistermans et al. (2023), which were obtained using the same protocol (see Supplementary Table S1 for a detailed overview).

Cestode genome assembly

We generated a reference genome assembly for the cestode *A. brevis*. We extracted high-molecular-weight DNA from a pool of 29 cestodes isolated from a single infected worker. An ultralow input library was prepared and sequenced using a PacBio Sequel II system. HiFi reads were called using DeepConsensus v1.2.0 (Baid et al., 2023) and assembled using Flye v2.9.2 (Kolmogorov et al., 2019), with the option "*–pacbio-raw*" to address sequence diversity within the sample, as well as options "*-i 4*," "*–no-alt-contigs*," and "*–scaffold*." The resultant assembly was deposited in the NCBI database under accession number JAUOZQ01. The assembly was annotated with BRAKER v3.0.3 (Brůna et al., 2021) in native mode using *A. brevis* RNA-seq data—from this study, Sistermans et al. (2023), and Stoldt et al. (2021)—and from all nuclear protein sequences of the Cyclophyllid family available on NCBI (accessed on August 13, 2023).

Mapping of RNA-seq reads

We mapped the cestode RNA reads using STAR v2.7.10b with default parameters (Dobin et al., 2013) onto a concatenated reference assembly, combining the cestode genome and the *T. nylanderi* genome assembly (Jongepier et al., 2022). This approach enabled us to filter out contaminant reads that mapped to the ant genome. We then constructed a gene count matrix using HTSeq-count (Putri et al., 2022) with default parameters including the discarding of multiply mapped. For functional annotation, we BLASTed the longest CDS isoform of each gene against the nonredundant invertebrate database (retrieved August 10, 2021) using BLAST diamond (Buchfink et al., 2014), and obtained Gene Ontology (GO) terms for these isoforms using eggNOG mapper against the eggNOG 5 database (Cantalapiedra et al., 2021).

For the ant RNA libraries, we first removed contaminant sequences from *A. brevis*, *Homo sapiens*, and *Escherichia coli*, as well as adaptors and vectors using FastQScreen v0.14.0 (Wingett & Andrews, 2018). The remaining sequences were trimmed down to 120 bp using fastp (Chen et al., 2018) with an N-base cutoff of 15 and mapped against a genome assembly of *T. nylanderi* (Jongepier et al., 2022) with HISAT2 v2.1.0 (Kim et al., 2015) using the "–downstream-transcriptome-assembly" parameter. We then obtained a genome-guided transcriptome assembly and gene count matrix with StringTie v1.3.6 (Pertea et al., 2015). Functional annotation was performed on the transcriptome assembly as described above for the cestode.

Differential gene expression analysis

We split the ant dataset into infected and uninfected samples, as the influence of parasite load has been analyzed elsewhere (Sistermans et al., 2023). We did Principal Component Analyses (PCAs) using DESeq2 (Love et al., 2014) in R (version 4.3.2) for the infected and uninfected workers separately to illustrate graphically the consequences of prevalence of infected workers and colony size on overall gene expression. For the infected ants, we fitted a negative binomial distribution using DESeq2. We performed a likelihood ratio test (LRT) linking gene expression to log-transformed infected worker prevalence (calculated as total number of infected workers divided by the total number of workers), using parasite load (number of cysticercoids per ant) and colony size (total number of workers in the colony) as reduced factors. Benjamini–Hochberg-adjusted *p*-values were extracted to identify differentially expressed genes (DEGs), the threshold for a DEG was a Benjamini–Hochberg-adjusted *p*-value of <0.05. We chose log-transformed values of infected worker prevalence to achieve a more even distribution of the prevalence data, minimizing the impact of individual outliers. The distribution after log transformation was closer to a normal distribution (Supplementary Figure S1). Additionally, we tested the effect of colony size by using log-transformed prevalence and parasite load as reduced factors. The influence of the interaction between log prevalence and colony size was assessed using log prevalence, parasite load, and colony ID as

reduced factors. We also examined overlapping DEGs across the three datasets by creating a Venn diagram using InteractiVenn (Heberle et al., 2015). To assess in which way transcriptomic shifts in response to infected worker prevalence were modified by colony size, we partitioned dataset into eight large colonies and seven small colonies and rerun LRTs with the log-transformed prevalence and a reduced factor of 1. Genes that were found to be significant in the interaction were removed from the main factor DEG lists. We divided DEGs identified in the main factor analyses into upregulated and downregulated genes and performed functional enrichments. First, we conducted a GO enrichment analysis with TopGO (Rahnenfuhrer, 2022) using Fisher's exact test and the weight01 algorithm. We decided not to apply multiple testing corrections to the GO terms, as the *p*-values obtained from this method are not independent. We considered only *p*-values below 0.01 in the GO enrichment analysis. We then applied the same analytical procedures to the cestode, and uninfected ant reads, with one exception. For the dataset of uninfected workers, some of which were sequenced in 2020 and others in 2022, we included sequencing year as a reduced factor in all models and assessed its influence using PCA (Supplementary Figure S2). We compared gene expression patterns between infected and uninfected workers as a function of the two main factors and their interactions, as well as the overlap of DEGs. It is important to note, however, that the models for the two datasets are not entirely identical. The number of cestodes was included in the model for the infected workers, while the year of sequencing was included for the uninfected workers. These differences may affect the analytical performance of the models and the results. Therefore, we report only on the overlap of DEGs but do not test whether it is larger than expected, as the use of different models leads to unclear expectations.

Survival analysis

We reanalyzed survival data from Beros et al. (2021) to assess the consequence of infected worker prevalence on age-specific mortality. These ant colonies were collected at the same sampling site as our ants for the transcriptome study and their survival had been analyzed in a period between 2014 and 2017. Day of death was available for queens and workers from 58 colonies up to 3 years after the beginning of the survival monitoring, from which point onwards individuals were considered censored (see Beros et al., [2021] for details on the monitoring procedure). We modeled the hazard rate of mortality using the spline-based survival model from Royston & Parmar (2002) with one knot parameter included in the package flexsurv (Jackson, 2016) in R (version 4.2.3). This model allows for more flexibility in modeling the hazard rate of mortality trajectories compared with usual parametric models such as Weibull or Gompertz models. We used three variables as covariates in the models. Colony size was defined as the number of individuals in a colony, Cat(Infected) was defined as a categorical variable informing whether a colony include at least one infected individual or not, and P(Infected) was defined as the proportion of infected individuals in a colony. The rationale behind two different variables relating to colony infection was to determine whether the infection effect was linked solely to the presence or absence of infected individuals, or whether mortality respond to the proportion of infected individuals.

Note that we performed our analyses independently for different categories of individuals, thus we estimated effects that are not simply the result of more individuals following mortality trajectories of infected individuals as infection prevalence increases. Analyses were performed independently for queens ($N = 58$), infected workers ($N = 103$), healthy nurses ($N = 285$), and healthy foragers ($N = 290$). In each case, the first set of models was run with all model combinations including colony size, P(Infected), and their interaction. A second set of models was then run for all individual types except for infected workers with all model combinations including colony size, Cat(infected), and their interaction. For each model, the value of the Akaike information criterion (AICc) was computed as a measure of the predictive ability of the model penalized for the number of parameters in the model and corrected for low sample size (Burnham & Anderson, 2004). Models were ranked according to their AICc values and the model with the lowest value was selected as the most parsimonious one. We also discuss the models within an AICc score of 2 to the top-ranked model with close predictive ability (see Supplementary Tables S2–S5 for AICc rankings).

Results

Changes in gene expression in the host with infected worker prevalence and colony size

The distribution of infected worker samples was mainly related to prevalence at the colony level (Figure 1A). The DESeq2 analysis identified 647 DEGs, whose expression in the fat body was linked to the prevalence of infected workers. In total, 339 genes were upregulated and 308 were downregulated with increasing prevalence. These DEGs showed 19 significantly enriched GO terms (Figure 1E), 11 and 8 for positively and negatively linked genes, respectively. Several of these GO terms are related to peptide and protein transport, including *peptide transport* (19 positively correlated genes) and *targeting of proteins to the ER* (five positively correlated genes). We also uncovered lipogenesis functions like *lipoxygenase pathway* and *lipoxin biosynthetic process* (each two positively correlated genes) and *negative regulation of fatty acid transport* (two negatively correlated genes). Shifts in fatty acid transport to mitochondria (Marshall et al., 2014) potentially indicate that infected workers alter their need for burning fat with increasing prevalence. The fat body tissue plays an important role in insect immunity (Hoffmann & Reichhart, 2002). Indeed, lipoxygenase products are important for eicosanoid signaling, involving the lipoxygenase pathway and lipoxin biosynthesis process (Kim & Stanley, 2021). In addition, other immune processes were involved, such as *IRES-dependent viral translation initiation* (two upregulated genes) and *positive regulation of NIK/NF-kappaB signaling* (three downregulated genes). The latter signaling process is implicated in the innate and adaptive immune system as a mediator of inflammatory responses (Liu et al., 2017). We plotted three differentially regulated genes and investigated their BLAST functions (Supplementary Figure S4) to examine whether starvation is responsible for the fat-burning

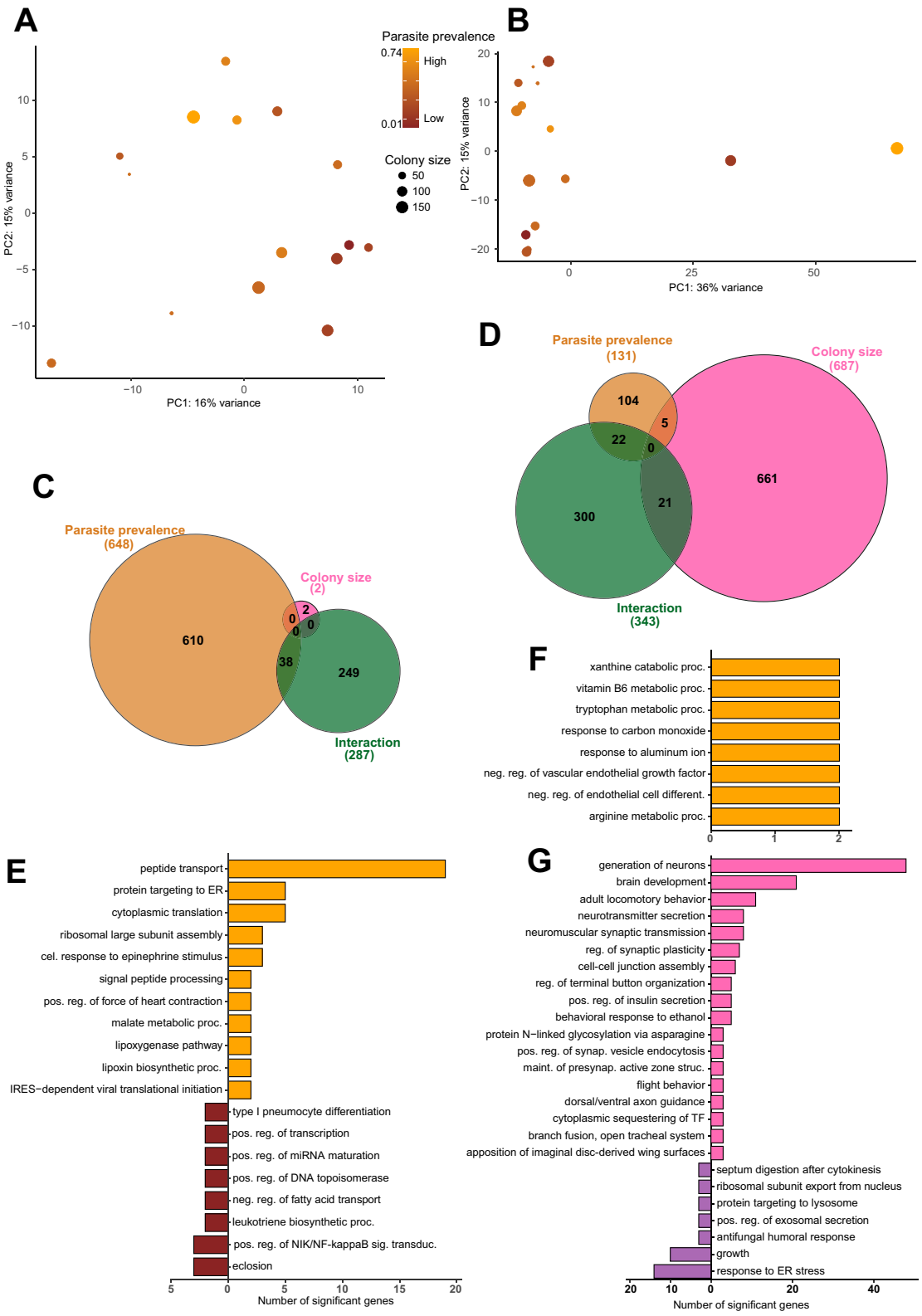

Figure 1. Ant fat body gene expression in relation to infection status of worker and prevalence of infected workers in the colony. (A) Principal component analysis of the infected ant transcriptome, where color signifies parasite prevalence with lighter colors representing more heavily infected colonies and dot size represents colony size with larger dots being from larger colonies. (B) Principal component analysis of the uninfected ant transcriptome. Venn diagrams showing the differentially expressed genes (DEGs) depending on prevalence of infected workers, colony size, and their interaction in (C) infected workers and (D) uninfected workers. (E) Gene Ontology (GO) terms for the infected ants for the prevalence analysis, where the orange bars to the right of the y-axis being the gene counts of upregulated DEGs and the brown bars on the left of the y-axis being the gene counts of downregulated DEGs. (F) GO terms for the uninfected ants for the parasite prevalence analysis, where we only found enrichments for the upregulated genes with parasite prevalence. (G) GO terms for the uninfected ants in the colony size analysis, where the hot pink bars on the right of the y-axis represent the genes upregulated with colony size and the purple bars on the left of the y-axis represent the genes downregulated with colony size.

physiological response. The first of these genes is *CCAAT/enhancer-binding protein* (Supplementary Figure S4A), which, when upregulated in high prevalence colonies, can protect cells against starvation (Lu et al., 2014). The encoded protein is also involved in protection against toxins from microbial pathogens (Reddy et al., 2016). A second gene, whose expression decreases with prevalence, *Zinc finger protein 662-like* (Supplementary Figure S4B), encodes for a protein that promotes stress-induced mitochondrial longevity (Merkwirth et al., 2017). Zinc finger nuclease cleavage of Bak and Bax proteins makes cells more resistant to starvation-induced apoptosis (Cost et al., 2010). Finally, *flavin reductase* (Supplementary Figure S4C), whose expression was decreased in high-prevalence colonies, indicates a lower stress tolerance, including resistance to starvation (Stegehake et al., 2013).

Colony size alone had little effect on gene expression in infected workers, as we found only two DEGs that were both downregulated with colony size, none of them overlapping with the DEGs for parasite prevalence (Figure 1C). One of them was annotated as a *facilitated trehalose transporter Tret1-2 homologue*, which is involved in cellular protection against desiccation (Kikawada et al., 2007). We identified 287 genes whose expression in infected workers was influenced by the interaction between colony size and prevalence. GO enrichment analyses did not uncover any terms with more than one gene being enriched. When we analyzed the DEGs for the effects of infected worker prevalence in small and large colonies, we identified only a single gene to be differentially expressed in the small colonies. This suggests that larger colonies can buffer the expression changes of this gene associated with infection prevalence. This gene did not yield an annotation in BLAST, but since we filtered out untranslatable transcripts in our filtering steps, it was clearly a gene.

The principal component analysis of uninfected worker samples revealed two outliers (Figure 1B). As neither our lab journal entries nor quality control of the RNA-seq samples explained their divergent gene expression, we found no external reason to remove them, and they remained in all subsequent analyses. However, to fairly assess the effect of these outliers, we did an additional analysis with outliers removed, which suggested that the effect of colony size hinges to some extend on the two outliers coming from very large colonies (see supplementary material).

The transcriptomes of uninfected workers were also influenced by the prevalence of infected workers in the colony. Here, 134 genes significantly changed their expression solely as a function of prevalence, with 54 and 82 being negatively and positively correlated, respectively. We found eight GO terms in the upregulated group with increasing prevalence, each represented by two genes (Figure 1F). At least three of these terms indicate stress responses, such as the response to aluminum ions, which is usually induced during aluminum stress, causing an accumulation of reactive oxygen species that destroy cell membrane integrity (Liu et al., 2022; Sujkowska-Rybkowska, 2012). Another term, *response to carbon monoxide* is also associated with oxidative stress, as CO poisoning is generally linked to lower hemocyanin levels (Baker & Wright, 1977). Finally, the term *xanthine catabolic process* indicates that genes involved in detoxification reactions changed their expression with prevalence (Battelli et al., 2016). Moreover, two DEGs each were linked to processes involved in the endothelium, namely *negative regulation of endothelial cell differentiation* and *negative regulation of vascular endothelial growth factor*, the latter appears to play a role in thoracic closure and insect immunity (Kipryushina et al., 2015).

Gene expression of uninfected workers was more strongly affected by colony size than that of infected workers. Here, we found 684 DEGs, of which 335 were downregulated with colony size and 349 were upregulated, 22 of the total DEGs were also differentially expressed with parasite prevalence (Figure 1D). GO enrichment analysis revealed 43 GO terms, so we only considered GO terms represented by three or more genes here, leaving 25 GO terms (Figure 1G). With colony size, many genes changed their expression with neuronal functions, including *neuron formation* (48 upregulated genes), *brain development* (21 upregulated genes), *adult locomotor behavior* (11 upregulated genes), *neurotransmitter secretion* (8 upregulated genes), *neuromuscular synaptic transmission* (8 upregulated genes), *regulation of synaptic plasticity* (7 upregulated genes), *behavioral response to ethanol* (5 upregulated genes), several other synaptic functions with 5 upregulated genes, and *flight behavior* (5 upregulated genes). These neurological functions can be prevalent as the fat body can promote the production of insulin-like peptides depending on when an insect last fed (Colombani et al., 2003; Skowronek et al., 2021). Unsurprisingly we uncover the *positive regulation of insulin secretion* (5 upregulated genes) for these DEGs as well. Other terms include *antifungal humoral response* with 3 downregulated genes, which is an immune function, and we found the GO term *growth* with 10 downregulated genes.

Our transcriptome analyses identified 343 genes whose expression was influenced by an interaction between colony size and prevalence of infected workers in the colony. GO enrichment analysis (Supplementary Figure S3A) revealed the number of these related to the defense against pathogens, e.g., *response to fungus* (7 genes), *antifungal humoral response* (4 genes), *positive regulation of NIK/NF-kappaB signal transduction* (3 genes), and *Toll-like receptor 9 signaling pathway* (2 genes). In total, 2 of the DEGs we found to be differentially expressed in the small colonies out of 17 genes and 1 of which in the large colonies out of 8 genes. We found functional annotation for two genes, one in the small colonies (considered buffered by colony size), was *phosphoinositide 3-kinase regulatory subunit 4-like*, which is involved in autophagy (Thoresen et al., 2010). The other gene (considered amplified by colony size) was *pheromone-binding protein Gp-9-like*, which has been described as a selfish gene in the fire ant *Solenopsis invicta* as it is involved in queen signaling and killing and is located on a social chromosome (Gotzek & Ross, 2009; Krieger & Ross, 2005; Wang et al., 2013).

A comparison of the influence of prevalence, colony size, and their interaction on the transcriptional activity in the fat body of infected and uninfected workers showed that infected workers were more influenced by the prevalence of infected workers in the colony, while uninfected workers were more affected by colony size. In both cases, the interaction between these two main factors was important. We found some overlap in the DEGs namely 10

for parasite prevalence, yet zero for colony size and eight for the interaction. As noted in the methods, this comparison should be taken with caution as the two DESeq2 models are not identical, with one including parasite load as a reduced factor and the other including sequencing year.

Changes in gene expression in the cestode with infected worker prevalence and colony size

The principal component analysis shows the data distribution as a function of prevalence of infected workers and colony size (Figure 2A). When analyzing the effects of prevalence on parasitic cestodes, we found 64 DEGs, of which 18 were upregulated and 46 were downregulated. Among the GO terms, we found only three for the downregulated genes (Figure 2C), namely *positive regulation of cell motility* (three genes), *sensory perception of pain* (three genes), and *positive regulation of dendrite extension* (two genes). Except for the regulation of cell motility, which is a subset of locomotion, the terms are associated with the nervous system.

Regarding the effect of colony size on cestode gene expression, we found 116 DEGs, 74 of these genes were downregulated and 42 were upregulated. We only found three genes overlapping with parasite prevalence (Figure 2B). Among the downregulated genes, we encountered a single enriched GO term, namely *lipid phosphorylation* with two representative genes. Phospholipids are important energy sources for developing platyhelminths (Ghosh & Misra, 2014; Humiczewska & Rajski, 2005), and therefore the differential lipid phosphorylation might be a result of *A. brevis* food intake depending on the colony size of their hosts.

The interaction between prevalence and colony size influenced the expression of 74 DEGs, for which we found two GO terms with two representative genes (Supplementary Figure S3B). The relevant GO terms included *sensory perception of pain* and *positive regulation of neuron apoptotic process*, both associated with neurological functions. Separate analyses for small and large colonies highlighted a prevalence effect, particularly with 1 DEG in small colonies and 15 DEGs in large colonies. Notably, there was no overlap in DEGs identified between the colony size groups in response to prevalence (Figure 2B), underscoring distinct transcriptional responses dependent on colony size.

Survival analysis

For queens, the model including only prevalence of infected workers (P[Infected]) as a covariate was selected as the most parsimonious one (Supplementary Table S2). Queens in colonies with a high proportion of infected individuals suffer from higher mortality during life: Increasing P(Infected) by 1% corresponded to an increase of the hazard rate of mortality by 9.07% (95% CI = [3.97;14.4]; Figure 3A). The second ranked model (ΔAICc = 0.32) also included a negative effect of colony size (increasing colony size by 1 corresponded to a decrease of the hazard rate of mortality by 0.50%; 95% CI = [0.00;1.22]) in addition to the effect of P(infected), hinting for a possible influence beneficial effect of larger colonies on queen mortality.

For infected workers, the selected model included colony size and P(Infected) as explanatory variables (Supplementary Table S3). Colony size led to diminished mortality of infected workers (increasing colony size by 1 corresponded to a decrease of the hazard rate of mortality by 0.60%; 95% CI = [0.10;1.01]), while an increasing proportion of infected individuals increased the mortality of infected workers (increasing P[Infected] by 1% corresponded to an increase of the hazard rate of mortality by 5.00%; 95% CI = [2.06;10.10]; Figure 3B). The second-ranked model (ΔAICc = 1.74) additionally included the interaction between P(infected) and colony size, but as it keeps the main effects in the model, the main effects are therefore a robust finding.

For healthy nurses, the top-ranked model included the category of infection as a covariate (hazard rate of mortality increased by 26.25%; 95% CI = [−0.12;59.59] in infected colonies compared with noninfected ones; Supplementary Table S4 and Figure 3C). Three alternative models were within ΔAICc < 2: P(infected) was included in the second-best model (ΔAICc = 0.94), Cat(infected) and colony size in the third one (ΔAICc = 1.47), and no covariate for the fourth one (ΔAICc = 1.74). The inclusion of a model with no effect among the four best models (all with ΔAICc < 2) means that our finding of higher mortality for nurses in infected colonies is on less solid footing than our other findings. While infection covariates were present in three out of the four top-ranked models as well as being selected in the top-ranked model, the evidence remains ambiguous for this class of workers.

For healthy foragers, the top-ranked model included P(infected), colony size, and their interaction (Supplementary Table S5). P(infected) had a positive effect on worker mortality and this effect was stronger in larger colonies: Increasing P(Infected) by 1% corresponded to an increase of the hazard rate of mortality by 1.94% (95% CI = [−0.31;4.25]) for a colony size of 75 while increasing P(Infected) by 1% corresponded to an increase of the hazard rate of mortality by 4.58% (95% CI = [1.14;6.92]) for a colony size of 150 (Figure 3D). Three other alternative models to predict healthy forager mortality were sufficiently highly ranked to merit discussing. The second-best model included only the effect of P(infected) and colony size without interaction (ΔAICc = 0.49), the third best one included the effect of Cat(infected) (ΔAICc = 1.52) and colony size while the third one included only the effect of Cat(infected) (ΔAICc = 1.62). All top-ranked models included at least one infection effect and most of them included colony size as a covariate. The evidence for infection impacting forager mortality is thus more solid than it is for nurses.

Discussion

Our survival and transcriptome analyses demonstrate that the prevalence of cestode-infected workers and colony size significantly affect the physiological activity and survival rates of both infected hosts and their healthy nestmates. Queen and infected worker mortality increases with the prevalence of infected workers in the colony. Furthermore, colony size alone and in conjunction with infected worker prevalence shapes gene expression patterns not only in infected and uninfected workers but also in the parasite itself.

As infected workers are often even better cared for than the queen (Beros et al., 2021), we hypothesized that both

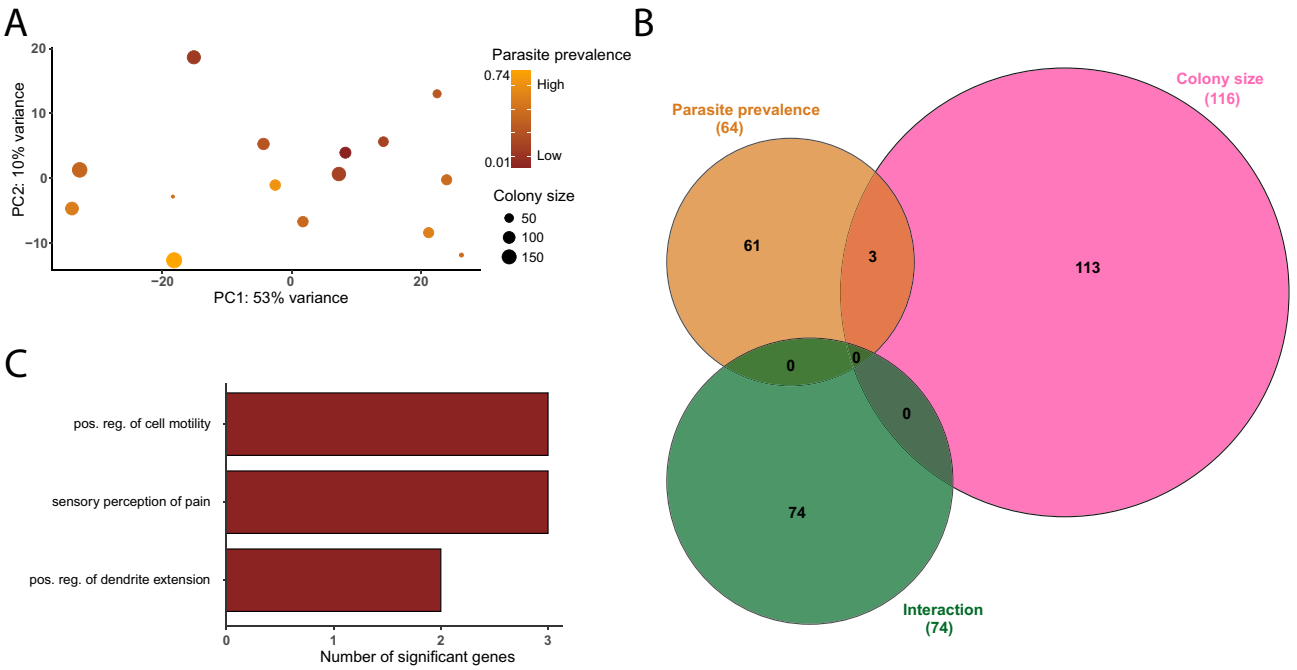

Figure 2. Cestode gene expression in relation to prevalence of infected workers in the colony. (A) Principal component analysis of the cestode transcriptomes, where color signifies prevalence. Lighter colors are from cestodes from colonies with a higher prevalence of infected workers. Dot size signifies colony size with larger dots representing larger colonies. (B) Venn diagram of differentially expressed genes (DEGs) with parasite prevalence, colony size, and interaction between both factors, circle size being representative of the number of DEGs. (C) Significant Gene Ontology (GO) terms in the cestode DEGs for prevalence. We only found GO terms for downregulated genes.

infected and uninfected nestmates, as well as the parasites themselves, will exhibit increased stress with a higher prevalence of infected workers, reflected in their transcriptional profiles. This hypothesis was supported by our findings: Survival analysis revealed elevated mortality rates with increasing parasite prevalence, and gene expression analysis identified 647 DEGs. Enrichment analyses suggest that this response stems from stress related to nutrient limitation, as we observed upregulation of several genes involved in lipogenesis pathways, indicative of a starvation response (Whitehouse & Tisdale, 2001). Additionally, several transport genes were differentially expressed, of which peptide transport has been linked to starvation in multiple systems (Ogihara et al., 1999; Tian et al., 2015; Vazquez et al., 1985; Voigt et al., 2006). Peptide transport is also associated with lipid metabolism, supporting a differential lipogenesis (Toprak, 2020). We also identified modulators of host immunity to parasites such as nematodes (Harcus et al., 2004), including IRES-dependent viral translational initiation and positive regulation of NIK/NF-kappaB signal transduction. The latter signal transduction cascade can cause weak immune reactions in parasitized organisms (Ebner et al., 2018). The pathway directionality, as indicated by the GO term, suggests a weakened immune response in workers from high-prevalence colonies. This may result from starvation stress reducing immunocompetence, impairing their ability to counter parasitic threats. However, causality remains unclear; low initial immunocompetence could have increased susceptibility to infection. An alternative, non-mutually exclusive explanation is heightened parasite virulence in heavily infected colonies, driven by increased resource extraction from nutritionally stressed hosts. Surprisingly, gene functions showed no evidence that cestodes in heavily infected colonies experience nutritional shortages. Instead, the parasites seem to secure sufficient resources by intensifying extraction from their hosts. Parasite transcriptomes support this, showing DEGs involved in parasite–host communication in response to infection prevalence. A similar phenomenon has been observed in nematodes, where host starvation slightly increased parasite lipid content (Beames et al., 1967; Smith et al., 1996). If a similar process occurs in the cestodes studied here, their increased effort to extract lipids from hosts could explain the observed downregulation of genes involved in fatty acid transport with increasing prevalence. Confirmation of this mechanism would require analyzing lipid content in hosts and parasites relative to the proportion of infected workers in a colony.

Cestodes are enveloped in a mucin layer within their hosts, which serves to shield them from the host's immune responses. However, this mucin layer also contains proteins that can stimulate dendrite maturation, potentially influencing host–parasite interactions at the cellular level (Casaravilla et al., 2014). This process seems to also inhibit the immune reaction of the host, creating conditions where a neuro secretory pathway is heavily involved in parasite–host interactions (Biserova et al., 2023). This may help explain the presence of two other neurological processes identified in our data: cell motility and sensory perception of pain. Additionally, gut-based parasites often exploit neurological pathways to extract resources, leveraging the properties of the enteric nervous system (Halliez & Buret, 2015).

In uninfected ants, we expected elevated stress and a higher risk of starvation in high-prevalence colonies.

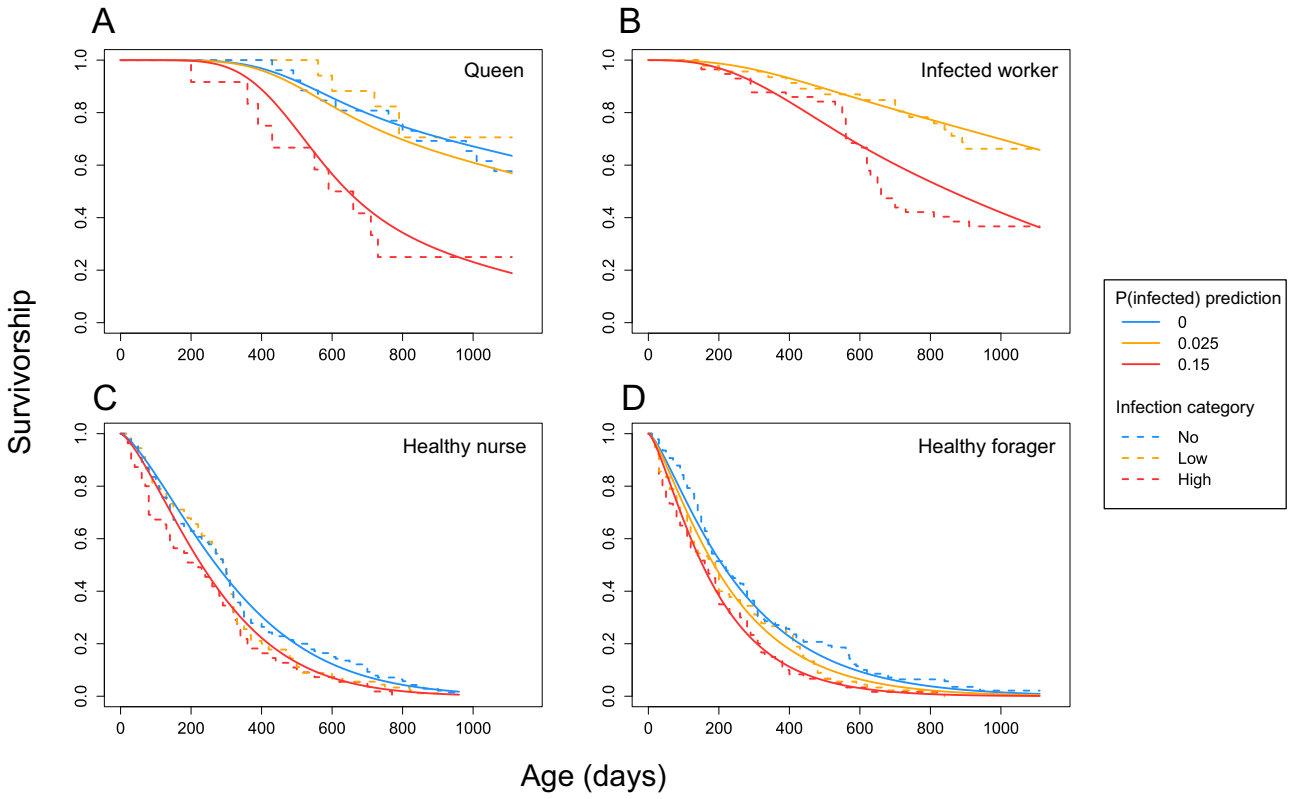

Figure 3. Age-specific survivorship of (A) queens, (B) infected workers, (C) healthy nurses, and (D) healthy foragers. For each plot, Kaplan–Meier curves are presented with dashed lines, in blue for individuals in colonies without infected individuals, in orange for individuals in colonies with low proportion of infected individuals (percentage of infected individuals is lower than 5%), and in red for individuals in colonies with high proportion of infected individuals (percentage of infected individuals is higher than 5%). Solid lines represent the predictions associated with the most parsimonious model for different proportion of infected individuals standardized for average colony size of each infection group, 0 in blue, 2.5% in orange, and 15% in red. For the prediction of healthy nurses, the red solid line corresponds to the predictions associated with the category having at least one infected individual in the colony and the blue solid line to the category without infected individual in the colony.

However, transcriptional shifts in uninfected workers were notably lower than in infected workers, with DEG counts slightly over one-third of those observed in infected ants. Survival analysis provided stronger evidence for foragers, rather than nurses, experiencing mortality costs in colonies with high infection prevalence. Yet, foragers were not included in our transcriptome analyses, preventing a direct comparison of their responses to those of nurses. Our findings align with a symptom of colony collapse disorder (CCD) in honeybees, namely the increased loss of foragers with pathogen pressure (Perry et al., 2015). This leads to increased recruitment of young foragers who are not adequately prepared for this task, which in turn makes them more likely to be lost to the colony (Dornberger et al., 2012). If this is also the case in our ants, we should be able to see a transcriptomic signal in nurses of the high-prevalence colonies to develop into foragers. The fat body may not be the ideal tissue to observe expression changes associated with such tasks switches, which may be more evident in the brain and antennae (Caminer et al., 2023). We did detect expression changes in the *regulation of endothelial growth factor*, which could be important for division of labor, since endothelial cells play a key role in the juvenile hormone degradation pathway in ants that switch their task from nurse to forager (Ju et al., 2023). A high prevalence of infected workers could lead to a syndrome in *T. nylanderi* that is comparable to CCD in honeybees. This colony collapse, also driven by increased mortality of the queen, could explain why highly infected colonies are so rare in the field. One plausible explanation for foragers' increased sensitivity to infection prevalence is that colony-level food stress may primarily impact those responsible for gathering food. Alternatively, the foraging workforce may predominantly consist of older workers, who are inherently more vulnerable (Beros et al., 2017; Blanchard et al., 2000). Young, uninfected nurses are better equipped to cope with the stress of cohabitating with many cestode-infected nestmates. Even so, functional analysis of DEGs in uninfected nurses revealed several stress responses, particularly to oxidative stress, with prominent responses to carbon monoxide and aluminum ions. Oxidative stress has multiple underlying causes; for instance, uninfected nurses exhibit increased activity levels in response to the presence of infected individuals (McArdle & Jackson, 2000); they experience nutrient deprivation (Morales et al., 2004) or mount an active immune response as through trophallactic exchange they detect the presence of the parasite (Negroni et al., 2020). Of these three, we regard the immune response to be the most likely, since the presence of pathogens in an ant colony have been previously shown to elicit an immune reaction in uninfected nestmates (Konrad et al., 2012). Nutrient deprivation may also in part be due to the activation of the immune system,

which is known to consume a lot of energy (Dolezal et al., 2019).

We also tested for the effects of colony size, as a given proportion of infection may impact small and large colonies differently, particularly since larger colonies tend to be older (Tschinkel, 1988). Intriguingly, cestode transcriptomes were more influenced by colony size than by infected worker prevalence, indicating that parasite physiology may be shaped by colony resources or host age. Specifically, colony size affected lipid phosphorylation in cestodes. Survival analyses revealed that colony size significantly improved the survival rate of infected workers, but no link was found between parasite prevalence and this metric, suggesting that parasite prevalence effects were not differentially experienced by larger or smaller colonies. The weak transcriptomic signal associated with survival effects of colony size likely stems from the use of younger ants in transcriptomic analyses, as age-specific signals may be more pronounced in older infected ants. While determining if an infected ant is nearing death remains challenging, we identified one desiccation-associated gene responsive to colony size that may serve as a primary transcriptomic marker for this effect. Additionally, the data suggest that cestodes depend on stored metabolic reserves.

The most substantial effect of colony size, however, was observed in uninfected ants, partly due to two outlier samples from the largest colonies, as detailed in the supplementary material. This may suggest that in very large colonies, transcriptional activity in the fat body of workers shifts. The genes affected by colony size appear to fulfill neurological functions. We interpret this as a homeostatic response, particularly considering the developmental functions associated with the DEGs identified after removing the two outliers, considering that our analysis focused on the fat body rather than the brain. The identification of hormonal functions, such as the positive regulation of insulin secretion, alongside neurological functions linked to development and growth rather than exclusively to signal transduction, implies a complex regulatory mechanism. Given the ongoing communication between the fat body and brain, the presence of signal transduction functions in the fat body is not unexpected (Skowronek et al., 2021). What the brain and the fat body communicate about is largely unknown aside from the production of insulin, which is heavily involved in feeding behavior and locomotion in insects (Erion & Sehgal, 2013). Likewise, we found a positive interaction between colony size and prevalence on survival, survival of foragers living in bigger colonies were more affected by the proportion of infected. Finally, growth and immunity may be influenced by colony size, which contrasts with previous findings indicating that colony size does not predict investment in antimicrobial defenses (Penick et al., 2018).

Conclusion

Our study demonstrates that infected worker prevalence exerts colony-wide effects, impacting not only infected individuals but also their healthy nestmates, the parasite itself, and even queen mortality. In this monogynous species, queen loss can lead to colony collapse, underscoring the potential severity of these effects. Such colony-wide impacts are rarely addressed in parasitism studies. Although the group integration seen in social insects may be less pronounced in other organisms, communication between healthy and infected individuals likely plays a critical role in buffering against fitness costs associated with parasitism. Moreover, the observed influence of colony size on the transcriptional activity of both healthy ants and the parasite highlights the importance of social structure in modulating host–parasite interactions.

Supplementary material

Supplementary material is available online at *Evolution*.

Data availability

Raw reads used for this study are available on the SRA under the BioProject ID: PRJNA1246159; for four samples (B6F, GXF, S5F, and T11F), we used reads published in Sistermans et al. (2023) under the BioProject ID: PRJNA950591. The genome assembly of *Anomotaenia brevis* can be found on NCBI under the ID: GCA_030710315.1. Data used for the survival analysis were published in Beros et al. (2021) and are available on Dryad under the DOI: 10.5061/dryad.1jwstqjt6. R scripts for analysis of both transcriptome data and survival data, gene count matrices, blast hits, enrichment analysis output, and the *A. brevis* genome assembly GTF file can be found on Dryad under the DOI: 10.5061/dryad.8cz8w9h3b.

Author contributions

Conceptualization of gene expression study—T.S., R.L., and S.F.; conceptualization of survival study—H.K. and V.R.; sampling—T.S., M.S., and S.F.; survival data observation and sampling—S.B.; transcriptomics experiments—T.S.; transcriptome analysis—T.S. with consultation of J.H. and M.S.; survival analysis—V.R. and H.K.; cestode genome generation—H.D. and J.H.; and original draft writing—T.S. with revision comments from H.K., V.R., H.D., R.L., and S.F.

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Conflict of interest

The authors declare no conflict of interest.

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References

Baid, G., Cook, D. E., Shafin, K., Yun, T., Llinares-López, F., Berthet, Q., Belyaeva, A., Töpfer, A., Wenger, A. M., Rowell, W. J., Yang, H., Kolesnikov, A., Ammar, W., Vert, J. P., Vaswani, A., McLean,

C. Y., Nattestad, M., Chang, P. C., & Carroll, A. (2023). DeepConsensus improves the accuracy of sequences with a gap-aware sequence transformer. *Nature Biotechnology*, *41*, 232–238. https://doi.org/10.1038/s41587-022-01435-7

Baker, G. M., & Wright, E. A. (1977). Effects of carbon monoxide on insects. *Bulletin of Environmental Contamination & Toxicology*, *17*(1), 98–104.

Battelli, M. G., Polito, L., Bortolotti, M., & Bolognesi, A. (2016). Xanthine oxidoreductase in drug metabolism: Beyond a role as a detoxifying enzyme. *Current Medicinal Chemistry*, *23*(35), 4027–4036. https://doi.org/10.2174/0929867323666160725091915

Beames, C. G., Harris, B. G., & Hopper, F. A. (1967). The synthesis of fatty acids from acetate by intact tissue and muscle extract of *Ascaris lumbricoides suum*. *Comparative Biochemistry and Physiology*, *20*(2), 509–521. https://doi.org/10.1016/0010-406X(67)90265-4

Beros, S., Foitzik, S., & Menzel, F. (2017). What are the mechanisms behind a parasite-induced decline in nestmate recognition in ants? *Journal of Chemical Ecology*, *43*(9), 869–880. https://doi.org/10.1007/s10886-017-0880-6

Beros, S., Jongepier, E., Hagemeier, F., & Foitzik, S. (2015). The parasite's long arm: A tapeworm parasite induces behavioural changes in uninfected group members of its social host. *Proceedings of the Royal Society B: Biological Sciences*, *282*(1819), 20151473. https://doi.org/10.1098/rspb.2015.1473

Beros, S., Lenhart, A., Scharf, I., Negroni, M. A., Menzel, F., & Foitzik, S. (2021). Extreme lifespan extension in tapeworm-infected ant workers. *Royal Society Open Science*, *8*(5), 202118. https://doi.org/10.1098/rsos.202118

Biserova, N. M., Kutyrev, I. A., Saitov, V. R., & Kolesnikov, I. A. (2023). The neuro-exocrine secretion: A new type of gland in tapeworms? *Zoology*, *160*(10), 126119. https://doi.org/10.1016/j.zool.2023.126119

Blanchard, G. B., Orledge, G. M., Reynolds, S. E., & Franks, N. R. (2000). Division of labour and seasonality in the ant *Leptothorax albipennis*: Worker corpulence and its influence on behaviour. *Animal Behaviour*, *59*(4), 723–738. https://doi.org/10.1006/anbe.1999.1374

Brůna, T., Hoff, K. J., Lomsadze, A., Stanke, M., & Borodovsky, M. (2021). BRAKER2: Automatic eukaryotic genome annotation with GeneMark-EP+ and AUGUSTUS supported by a protein database. *NAR Genomics and Bioinformatics*, *3*(1), 1–11. https://doi.org/10.1093/nargab/lqaa108

Buchfink, B., Xie, C., & Huson, D. H. (2015). Fast and sensitive protein alignment using DIAMOND. *Nature Methods*, *12*(1), 59–60. https://doi.org/10.1038/nmeth.3176

Burnham, K. P., & Anderson, D. R. (Eds.). (2004). *Model selection and multimodel inference*. Springer. https://doi.org/10.1007/b97636

Caminer, M. A., Libbrecht, R., Majoe, M., Ho, D. V., Baumann, P., & Foitzik, S. (2023). Task-specific odorant receptor expression in worker antennae indicates that sensory filters regulate division of labor in ants. *Communications Biology*, *6*(1), 1–12. https://doi.org/10.1038/s42003-023-05273-4

Cantalapiedra, C. P., Hernn̨andez-Plaza, A., Letunic, I., Bork, P., & Huerta-Cepas, J. (2021). eggNOG-mapper v2: Functional annotation, orthology assignments, and domain prediction at the metagenomic scale. *Molecular Biology and Evolution*, *38*(12), 5825–5829. https://doi.org/10.1093/molbev/msab293

Casaravilla, C., Pittini, Á., Rückerl, D., Seoane, P. I., Jenkins, S. J., Macdonald, A. S., Ferreira, A. M., Allen, J. E., & Diaz, Á. (2014). Unconventional maturation of dendritic cells induced by particles from the laminated layer of larval *Echinococcus granulosus*. *Infection and Immunity*, *82*(8), 3164–3176. https://doi.org/10.1128/IAI.01959-14

Chapuisat, M., Oppliger, A., Magliano, P., & Christe, P. (2007). Wood ants use resin to protect themselves against pathogens. *Proceedings of the Royal Society B: Biological Sciences*, *274*(1621), 2013–2017. https://doi.org/10.1098/rspb.2007.0531

Chen, S., Zhou, Y., Chen, Y., & Gu, J. (2018). fastp: An ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics*, *34*(17), i884–i890. https://doi.org/10.1093/bioinformatics/bty560

Colombani, J., Raisin, S., Pantalacci, S., Radimerski, T., Montagne, J., & Léopold, P. (2003). A nutrient sensor mechanism controls *Drosophila* growth. *Cell*, *114*(6), 739–749. https://doi.org/10.1016/S0092-8674(03)00713-X

Cost, G. J., Freyvert, Y., Vafiadis, A., Santiago, Y., Miller, J. C., Rebar, E., Collingwood, T. N., Snowden, A., Gregory, P. D., Biosciences, S., & Boulevard, C. (2010). BAK and BAX deletion using Zinc-finger nucleases yields apoptosis-resistant CHO cells. *Biotechnology and Bioengineering*, *105*(2), 330–340. https://doi.org/10.1002/bit.22541

Cremer, S., Armitage, S. A. O., & Schmid-Hempel, P. (2007). Social immunity. *Current Biology*, *17*(16), R693–R702. https://doi.org/10.1016/j.cub.2007.06.008

Cremer, S., Pull, C. D., & Fürst, M. A. (2018). Social immunity: Emergence and evolution of colony-level disease protection. *Annual Review of Entomology*, *63*, 105–123. https://doi.org/10.1146/annurev-ento-020117-043110

Crowl, T. A., Crist, T. O., Parmenter, R. R., Belovsky, G., & Lugo, A. E. (2008). The spread of invasive species and infectious disease as drivers of ecosystem change. *Frontiers in Ecology and the Environment*, *6*(5), 238–246. https://doi.org/10.1890/070151

Dobin, A., Davis, C. A., Schlesinger, F., Drenkow, J., Zaleski, C., Jha, S., Batut, P., Chaisson, M., & Gingeras, T. R. (2013). STAR: Ultrafast universal RNA-seq aligner. *Bioinformatics*, *29*(1), 15–21. https://doi.org/10.1093/bioinformatics/bts635

Dolezal, T., Krejcova, G., Bajgar, A., Nedbalova, P., & Strasser, P. (2019). Molecular regulations of metabolism during immune response in insects. *Insect Biochemistry and Molecular Biology*, *109*, 31–42. https://doi.org/10.1016/j.ibmb.2019.04.005

Dornberger, L., Mitchell, C., Hull, B., Ventura, W., Shopp, H., Kribs-Zaleta, C., Kojouharov, H., & Grover, J. (2012). *Death of the bees: A mathematical model of colony collapse disorder*. Mathematics Preprint Series. Technical Report. The University of Texas. https://citeseerx.ist.psu.edu/document?repid=rep1&type=pdf&doi=a42f672d83727b90aa8109c86c71a1b31632203c

Ebner, F., Kuhring, M., Radonic, A., Midha, A., Renard, B. Y., & Hartmann, S. (2018). Silent witness: Dual-species transcriptomics reveals epithelial immunological quiescence to helminth larval encounter and fostered larval development. *Frontiers in Immunology*, *9*(August), 1–15. https://doi.org/10.3389/fimmu.2018.01868

Erion, R., & Sehgal, A. (2013). Regulation of insect behavior via the insulin-signaling pathway. *Frontiers in Physiology*, *4*, 353. https://doi.org/10.3389/fphys.2013.00353

Feldmeyer, B., Mazur, J., Beros, S., Lerp, H., Binder, H., & Foitzik, S. (2016). Gene expression patterns underlying parasite-induced alterations in host behaviour and life history. *Molecular Ecology*, *25*(2), 648–660. https://doi.org/10.1111/mec.13498

Forfert, N., Natsopoulou, M. E., Frey, E., Rosenkranz, P., Paxton, R. J., & Moritz, R. F. A. (2015). Parasites and pathogens of the honeybee (*Apis mellifera*) and their influence on inter-colonial transmission. *PLoS ONE*, *10*(10), e0140337. https://doi.org/10.1371/journal.pone.0140337

Fries, I., & Camazine, S. (2001). Implications of horizontal and vertical pathogen transmission for honey bee epidemiology. *Apidologie*, *32*(3), 199–214. https://doi.org/10.1051/apido:2001122

Ghosh, D., & Misra, K. K. (2014). Comparison of fatty acid contents of the neutral and phospholipids of the trematode *Paramphistomum cervi* and liver of its host, *Capra hircus*. *Journal of Parasitic Diseases*, *38*(2), 223–232. https://doi.org/10.1007/s12639-012-0229-6

Gotzek, D., & Ross, K. G. (2009). Current status of a model system: The gene Gp-9 and its association with social organization in fire ants. *PLoS ONE*, *4*(11), e7713. https://doi.org/10.1371/journal.pone.0007713

Granroth-Wilding, H. M. V., Burthe, S. J., Lewis, S., Herborn, K. A., Takahashi, E. A., Daunt, F., & Cunningham, E. J. A. (2015). Indirect

effects of parasitism: Costs of infection to other individuals can be greater than direct costs borne by the host. *Proceedings of the Royal Society B: Biological Sciences*, *282*(1811), 20150602. https://doi.org/10.1098/rspb.2015.0602

Halliez, M. C. M., & Buret, A. G. (2015). Gastrointestinal parasites and the neural control of gut functions. *Frontiers in Cellular Neuroscience*, *9*, 452. https://doi.org/10.3389/fncel.2015.00452

Harcus, Y. M., Parkinson, J., Fernández, C., Daub, J., Selkirk, M. E., Blaxter, M. L., & Maizels, R. M. (2004). Signal sequence analysis of expressed sequence tags from the nematode *Nippostrongylus brasiliensisand* the evolution of secreted proteins in parasites. *Genome Biology*, *5*(6), R39. https://doi.org/10.1186/gb-2004-5-6-r39

Hart, B. L., & Hart, L. A. (2018). How mammals stay healthy in nature: The evolution of behaviours to avoid parasites and pathogens. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *373*(1751), 20170205. https://doi.org/10.1098/rstb.2017.0205

Hartke, J., Ceron-Noriega, A., Stoldt, M., Sistermans, T., Kever, M., Fuchs, J., Butter, F., & Foitzik, S. (2023). Long live the host! Proteomic analysis reveals possible strategies for parasitic manipulation of its social host. *Molecular Ecology*, *32*(21), 5877–5889. https://doi.org/10.1111/mec.17155

Heberle, H., Meirelles, V. G., da Silva, F. R., Telles, G. P., & Minghim, R. (2015). InteractiVenn: A web-based tool for the analysis of sets through Venn diagrams. *BMC Bioinformatics*, *16*(1), 1–7. https://doi.org/10.1186/s12859-015-0611-3

Hoffmann, J. A., & Reichhart, J. (2002). Drosophila innate immunity: An evolutionary perspective. *Nature Immunology*, *3*(2), 121–126. https://doi.org/10.1038/ni0202-121

Hudson, P. J., Dobson, A. P., & Lafferty, K. D. (2006). Is a healthy ecosystem one that is rich in parasites? *Trends in Ecology & Evolution*, *21*(7), 381–385. https://doi.org/10.1016/j.tree.2006.04.007

Humiczewska, M., & Rajski, K. (2005). Lipids in the host-parasite system: Digestive gland of *Lymnaea truncatula* infected with the developmental stages of *Fasciola hepatica*. *Acta Parasitologica*, *50*(3), 235–239.

Jackson, C. H. (2016). flexsurv: A platform for parametric survival modeling. *Journal of Statistical Software*, *70*(8), i08. https://doi.org/10.18637/jss.v070.i08

Jongepier, E., Séguret, A., Labutin, A., Feldmeyer, B., Gstöttl, C., Foitzik, S., Heinze, J., & Bornberg-Bauer, E. (2022). Convergent loss of chemoreceptors across independent origins of slave-making in ants. *Molecular Biology and Evolution*, *39*(1), msab305. https://doi.org/10.1093/molbev/msab305

Ju, L., Glastad, K. M., Sheng, L., Gospocic, J., Kingwell, C. J., Davidson, S. M., Kocher, S. D., Bonasio, R., & Berger, S. L. (2023). Hormonal gatekeeping via the blood–brain barrier governs caste-specific behavior in ants. *Cell*, *186*(20), 4289–4309.e23. https://doi.org/10.1016/j.cell.2023.08.002

Kikawada, T., Saito, A., Kanamori, Y., Nakahara, Y., Iwata, K. I., Tanaka, D., Watanabe, M., & Okuda, T. (2007). Trehalose transporter 1, a facilitated and high-capacity trehalose transporter, allows exogenous trehalose uptake into cells. *Proceedings of the National Academy of Sciences*, *104*(28), 11585–11590. https://doi.org/10.1073/pnas.0702538104

Kim, D., Langmead, B., & Salzberg, S. L. (2015). HISAT: A fast spliced aligner with low memory requirements. *Nature Methods*, *12*(4), 357–360. https://doi.org/10.1038/nmeth.3317

Kim, Y., & Stanley, D. (2021). Eicosanoid signaling in insect immunology: New genes and unresolved issues. *Genes*, *12*(2), 211. https://doi.org/10.3390/genes12020211

Kipryushina, Y. O., Yakovlev, K. V., & Odintsova, N. A. (2015). Vascular endothelial growth factors: A comparison between invertebrates and vertebrates. *Cytokine & Growth Factor Reviews*, *26*(6), 687–695. https://doi.org/10.1016/j.cytogfr.2015.04.001

Kolmogorov, M., Yuan, J., Lin, Y., & Pevzner, P. A. (2019). Assembly of long, error-prone reads using repeat graphs. *Nature Biotechnology*, *37*(5), 540–546. https://doi.org/10.1038/s41587-019-0072-8

Konrad, M., Vyleta, M. L., Theis, F. J., Stock, M., Tragust, S., Klatt, M., Drescher, V., Marr, C., Ugelvig, L. V., & Cremer, S. (2012). Social transfer of pathogenic fungus promotes active immunisation in ant colonies. *PLoS Biology*, *10*(4), e1001300. https://doi.org/10.1371/journal.pbio.1001300

Krieger, M. J. B., & Ross, K. G. (2005). Molecular evolutionary analyses of the odorant-binding protein gene Gp-9 in fire ants and other *Solenopsis* species. *Molecular Biology and Evolution*, *22*(10), 2090–2103. https://doi.org/10.1093/molbev/msi203

Liu, H., Zhu, R., & Shu, K. (2022). Aluminum stress signaling, response, and adaptive mechanisms in plants. *Plant Signaling & Behavior*, *17*(1), 2057060. https://doi.org/10.1080/15592324.2022.2057060

Liu, T., Zhang, L., Joo, D., & Sun, S. C. (2017). NF-κB signaling in inflammation. *Signal Transduction and Targeted Therapy*, *2*(April), 17023. https://doi.org/10.1038/sigtrans.2017.23

Love, M. I., Huber, W., & Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology*, *15*, 550. https://doi.org/10.1186/s13059-014-0550-8

Lu, G., Ang, Y. H., Zhou, J., Tamilarasi, J., Yan, B., Lim, Y. C., Srivastava, S., Salto-tellez, M., Hui, K. M., Shen, H., Nguyen, L. N., Tan, B. C., Silver, D. L., & Hooi, S. C. (2015). CCAAT/enhancer binding protein a predicts poorer prognosis and prevents energy starvation–induced cell death in hepatocellular carcinoma. *Hepatology*, *61*(3), 965–978. https://doi.org/10.1002/hep.27593

McArdle, A., & Jackson, M. J. (2000). Exercise, oxidative stress and ageing. *Journal of Anatomy*, *197*(4), 539–541. https://doi.org/10.1046/j.1469-7580.2000.19740539.x

Marshall, W. J., Lapsley, M., Day, A. P., & Ayling, R. M. (2014). *Clinical biochemistry: Metabolic and clinical aspects*. Elsevier Health Sciences. https://doi.org/10.1016/C2011-0-07946-2

Merkwirth, C., Jovaisaite, V., Durieux, J., Matilainen, O., Sabine, D., Quiros, P. M., Steffen, K. K., Williams, E. G., Uhlein, S. N., Murillo, V., Wolff, S. C., Shaw, R. J., Auwerx, J., & Dillin, A. (2017). Two conserved histone demethylases regulate mitochondrial stress-induced longevity. *Cell*, *165*(5), 1209–1223. https://doi.org/10.1016/j.cell.2016.04.012.Two

Minaud, É., Rebaudo, F., Davidson, P., Hatjina, F., Hotho, A., Mainardi, G., Steffan-Dewenter, I., Vardakas, P., Verrier, E., & Requier, F. (2024). How stressors disrupt honey bee biological traits and overwintering mechanisms. *Heliyon*, *10*(14), e34390. https://doi.org/10.1016/j.heliyon.2024.e34390

Morales, A. E., Pe, A., Abella, E., & Cardenete, G. (2004). Oxidative stress and antioxidant defenses after prolonged starvation in *Dentex dentex* liver. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, *139*, 153–161. https://doi.org/10.1016/j.cca.2004.10.008

Mouritsen, K. N., & Poulin, R. (2002). Parasitism, community structure and biodiversity in intertidal ecosystems. *Parasitology*, *124*(Suppl.), 101–117. https://doi.org/10.1017/s0031182002001476

Negroni, M. A., Macit, M. N., Stoldt, M., Feldmeyer, B., & Foitzik, S. (2021). Molecular regulation of lifespan extension in fertile ant workers. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *376*(1823), 20190736. https://doi.org/10.1098/rstb.2019.0736

Negroni, M. A., Segers, F. H. I. D., Vogelweith, F., & Foitzik, S. (2020). Immune challenge reduces gut microbial diversity and triggers fertility-dependent gene expression changes in a social insect. *BMC Genomics*, *21*, 816. https://doi.org/10.1186/s12864-020-07191-9

Odum, E. P., & Barrett, G. W. (1971). *Fundamentals of ecology*. Saunders.

Ogihara, H., Suzuki, T., Nagamachi, Y., Inui, K., & Takata, K. (1999). Peptide transporter in the rat small intestine: Ultrastructural localization and the effect of starvation and administration of amino acids. *The Histochemical Journal*, *31*, 169–174. https://doi.org/10.1023/A:1003515413550

Penick, C. A., Halawani, O., Pearson, B., Mathews, S., López-Uribe, M. M., Dunn, R. R., & Smith, A. A. (2018). External immunity in ant societies: Sociality and colony size do not predict investment in antimicrobials. *Royal Society Open Science*, *5*(2), 171332. https://doi.org/10.1098/rsos.171332

Perry, C. J., Søvik, E., Myerscough, M. R., & Barron, A. B. (2015). Rapid behavioral maturation accelerates failure of stressed honey bee colonies. *Proceedings of the National Academy of Sciences*, *112*(11), 3427–3432. https://doi.org/10.1073/pnas.1422089112

Pertea, M., Pertea, G. M., Antonescu, C. M., Chang, T. C., Mendell, J. T., & Salzberg, S. L. (2015). StringTie enables improved reconstruction of a transcriptome from RNA-seq reads. *Nature Biotechnology*, *33*(3), 290–295. https://doi.org/10.1038/nbt.3122

Plateaux, L. (1972). Sur les modifications produites chez une fourmi par la présence d'un parasite cestode. *Annales Des Sciences Naturelles*, *14*, 203–220.

Plateaux, L. (1986). Comparison des cycles saisonniers, des durees des societes et des production des trois especes de fourmis *Leptothorax* (Myrafant) du groupe nylanderi. *Actes Des Colloques Insectes Sociaux*, *3*, 221–234.

Price, P. W., Westoby, M., Rice, B., Atsatt, P. R., & Fritz, R. S. (1986). Parasite mediation in ecological interactions. *Annual Review of Ecology and Systematics*, *17*(1986), 487–505. https://doi.org/10.1146/annurev.es.17.110186.002415

Putri, G. H., Anders, S., Pyl, P. T., Pimanda, J. E., & Zanini, F. (2022). Gene expression analysing high-throughput sequencing data in Python with HTSeq 2.0. *Bioinformatics*, *38*(10), 2943–2945. https://doi.org/10.1093/bioinformatics/btac166

Rahnenfuhrer, A. (2022). topgo: Enrichment analysis for gene ontology. R package version 2.50.0. https://bioconductor.org/packages/topGO.

Reddy, K. C., Dunbar, T. L., Nargund, A. M., Haynes, C. M., Emily, R., Diego, C. S., Jolla, L., & Program, B. (2016). The *C. elegans* CCAAT-enhancer binding protein gamma is required for surveillance immunity. *Cell Reports*, *14*(7), 1581–1589. https://doi.org/10.1016/j.celrep.2016.01.055.The

Romano, V., Sueur, C., & MacIntosh, A. J. J. (2022). The trade-off between information and pathogen transmission in animal societies. *Oikos*, *2022*(10), e08290. https://doi.org/10.1111/oik.08290

Rowland, B. W., Rushton, S. P., Shirley, M. D. F., Brown, M. A., & Budge, G. E. (2021). Identifying the climatic drivers of honey bee disease in England and Wales. *Scientific Reports*, *11*, 21953. https://doi.org/10.1038/s41598-021-01495-w

Royston, P., & Parmar, M. K. B. (2002). Flexible parametric proportional-hazards and proportional-odds models for censored survival data, with application to prognostic modelling and estimation of treatment effects. *Statistics in Medicine*, *21*(15), 2175–2197. https://doi.org/10.1002/sim.1203

Scharf, I., Modlmeier, A. P., Beros, S., & Foitzik, S. (2012). Ant societies buffer individual-level effects of parasite infections. *The American Naturalist*, *180*(5), 671–683. https://doi.org/10.1086/667894

Shik, J. Z. (2010). The metabolic costs of building ant colonies from variably sized subunits. *Behavioral Ecology and Sociobiology*, *64*(12), 1981–1990. https://doi.org/10.1007/s00265-010-1009-x

Sinclair, A. R. E. (1979). The eruption of the ruminants. In A. R. E. Sinclair, & N. Norton-Griffiths (Eds.), *Serengetti: Dynamics of an ecosystem*(pp. 82–103). University of Chicago Press.

Sistermans, T., Hartke, J., Stoldt, M., Libbrecht, R., & Foitzik, S. (2023). The influence of parasite load on transcriptional activity and morphology of a cestode and its ant intermediate host. *Molecular Ecology*, *32*, 4412–4426. https://doi.org/10.1111/mec.16995

Skowronek, P., Wójcik, Ł., & Strachecka, A. (2021). Fat body-multifunctional insect tissue. *Insects*, *12*(6), 547. https://doi.org/10.3390/insects12060547

Smith, V. P., Selkirk, M. E., & Gounaris, K. (1996). Identification and composition of lipid classes in surface and somatic preparations of adult *Brugia malayi*. *Molecular and Biochemical Parasitology*, *78*(1–2), 105–116. https://doi.org/10.1016/S0166-6851(96)02615-1

Stegehake, D., Daniel, J., Drescher, M., Ajonina, I., Ajonina, C., Hertel, P., Woltersdorf, C., & Liebau, E. (2013). The glutathione reductase GSR-1 determines stress tolerance and longevity in *Caenorhabditis elegans*. *PLoS ONE*, *8*(4), e60731. https://doi.org/10.1371/journal.pone.0060731

Stock, M., Milutinović, B., Hoenigsberger, M., Grasse, A. V., Wiesenhofer, F., Kampleitner, N., Narasimhan, M., Schmitt, T., & Cremer, S. (2023). Pathogen evasion of social immunity. *Nature Ecology & Evolution*, *7*(3), 450–460. https://doi.org/10.1038/s41559-023-01981-6

Stoldt, M., Klein, L., Beros, S., Butter, F., Jongepier, E., Feldmeyer, B., & Foitzik, S. (2021). Parasite presence induces gene expression changes in an ant host related to immunity and longevity. *Genes*, *12*(1), 95. https://doi.org/10.3390/genes12010095

Stroeymeyt, N., Casillas-Pérez, B., & Cremer, S. (2014). Organisational immunity in social insects. *Current Opinion in Insect Science*, *5*(1), 1–15. https://doi.org/10.1016/j.cois.2014.09.001

Sujkowska-Rybkowska, M. (2012). Reactive oxygen species production and antioxidative defense in pea (*Pisum sativum L.*) root nodules after short-term aluminum treatment. *Acta Physiologiae Plantarum*, *34*, 1387–1400. https://doi.org/10.1007/s11738-012-0935-9

Thomas, F., & Bonsall, M. B. (2005). Parasitism, biodiversity and conservation. In F. Thomas, & F. Renaud (Eds.), *Parasitism and ecosystems*(pp. 124–139). Oxford University Press. https://doi.org/10.1093/acprof:oso/9780198529873.003.0009

Thoresen, S. B., Pedersen, N. M., Liestøl, K., & Stenmark, H. (2010). A phosphatidylinositol 3-kinase class III sub-complex containing VPS15, VPS34, Beclin 1, UVRAG and BIF-1 regulates cytokinesis and degradative endocytic traffic. *Experimental Cell Research*, *316*(20), 3368–3378. https://doi.org/10.1016/j.yexcr.2010.07.008

Tian, J., He, G., Mai, K., & Liu, C. (2015). Effects of postprandial starvation on mRNA expression of endocrine-, amino acid and peptide transporter-, and metabolic enzyme-related genes in zebrafish (*Danio rerio*). *Fish Physiology and Biochemistry*, *41*, 773–787. https://doi.org/10.1007/s10695-015-0045-x

Toprak, U. (2020). The role of peptide hormones in insect lipid metabolism. *Frontiers in Physiology*, *11*, 1–33. https://doi.org/10.3389/fphys.2020.00434

Tschinkel, W. R. (1988). Colony growth and the ontogeny of worker polymorphism in the fire ant, *Solenopsis invicta*. *Behavioral Ecology and Sociobiology*, *22*(2), 103–115. https://doi.org/10.1007/BF00303545

Vazquez, J. A., Morse, E. L., & Adibi, A. (1985). Effect of starvation on amino acid and peptide transport and peptide hydrolysis in humans. *The American Journal of Physiology*, *249*(5), G563–G566.

Vindas, M. A., Midttun, H. L. E., Nadler, L. E., Fontaine, R., Weltzien, F.-A., Øverli, Ø., & Johansen, I. B. (2023). Brain-infecting parasites leave lasting effects on behaviour even in resistant hosts. *Functional Ecology*, *37*(4), 852–859. https://doi.org/10.1111/1365-2435.14248

Voigt, B., Schweder, T., Sibbald, M. J. J. B., Albrecht, D., Ehrenreich, A., Bernhardt, J., Feesche, J., Maurer, K.-H., Gottschalk, G., Van Dijl, J. M., & Hecker, M. (2006). The extracellular proteome of *Bacillus licheniformis* grown in different media and under different nutrient starvation conditions. *Proteomics*, *6*, 268–281. https://doi.org/10.1002/pmic.200500091

Wang, J., Wurm, Y., Nipitwattanaphon, M., Riba-Grognuz, O., Huang, Y. C., Shoemaker, D., & Keller, L. (2013). A Y-like social chromosome causes alternative colony organization in fire ants. *Nature*, *493*(7434), 664–668. https://doi.org/10.1038/nature11832

Webber, Q. M. R., Fletcher, Q. E., & Willis, C. K. R. (2017). Viral richness is positively related to group size, but not mating system, in bats. *EcoHealth*, *14*(4), 652–661. https://doi.org/10.1007/s10393-017-1276-3

Whitehouse, A. S., & Tisdale, M. J. (2001). Downregulation of ubiquitin-dependent proteolysis by eicosapentaenoic acid in acute starvation. *Biochemical and Biophysical Research Communications*, *285*, 598–602. https://doi.org/10.1006/bbrc.2001.5209

Wingett, S. W., & Andrews, S. (2018). FastQ Screen: A tool for multi-genome mapping and quality control [version 1; referees: 3 approved, 1 approved with reservations]. *F1000Research*, *7*(May), 1338. https://doi.org/10.12688/f1000research.15931.1

Zavaleta, E., Pasari, J., Moore, J., Hernández, D., Suttle, K. B., & Wilmers, C. C. (2009). Ecosystem responses to community disassembly. *Annals of the New York Academy of Sciences*, *1162*(1), 311–333. https://doi.org/10.1111/j.1749-6632.2009.04448.x

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