Transcriptomic dynamics and interaction in a host and parasite system

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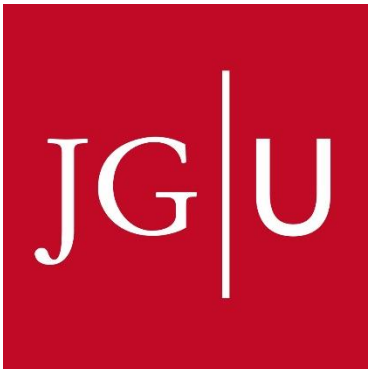

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Summary

Parasites have a profound impact on their hosts, sometimes even being able to manipulate their phenotype in order to increase the chances of transmission to the final host. This also seems to be the case with the cestode *Anomotaenia brevis* and their intermediate host, the ant *Temnothorax nylanderi*. When infected, we observe changes in the cuticle colour and sclerotization, activity, lifespan, interactions with nestmates and muscle mass. These changes co-occur with changes in gene expression in the ant, making it difficult to determine whether the cestode is truly the one pulling the strings in this interaction or whether phenotypic changes are simply side effects of cestode infection. In this dissertation I used various methods to investigate whether the parasite is truly the manipulator in this interaction or whether we can find alternative explanations for gene expression differences.

In the first chapter we investigated the effect of parasite load on this system. We compared the gene expression of uninfected, lowly infected and highly infected hosts as well as parasites that coinfect the host with few others and many others. We observed similar changes in parasite and host, where both suffered from higher stress and where the host seems to be more aware of the parasite with the parasite having a stronger need to evade the host's immune response.

In chapter 2 we decided to immune challenge ants with bacterial lipopolysaccharide in order to understand the difference between the immune reaction to the cestode and the immune reaction to the parasite on a gene expression level. We combined this by investigating the gut microbiome and see what both immune challenge (parasitic and mock bacterial) have on the host. We found a large pool of differentially expressed genes that also strongly impact the immune response. Upon deeper investigation we especially found that the genes that are differentially expressed upon both parasite infection and mock challenge are differentially expressed in different directions. Laying bare a possible immune system inhibition in this system. However, this inhibition did not affect the immune system against bacteria, meaning that the cestode is capable of minimizing the attacks against it while not compromising the ant's ability to fight other parasites. In the gut microbiome meanwhile we found a loss in biodiversity upon parasite infection and a dominance of intracellular groups in the infected ants, possibly hinting at an earlier immune reaction from cestode infection.

In the final chapter we tested what the effects of parasite prevalence are in this system on the gene expression by correlating gene expression against the proportion of infected ants in the colony in both infected hosts, uninfected hosts and parasites. Here we found a stress reaction in the ants while the parasite, though affected in its gene expression, does not seem to suffer under these conditions. We further employed a novel method to investigate the interactome between parasite and host where we shed new light upon the molecular interactions between parasite and host, here we found the parasite transcriptome to be highly interactive with that of the ant both in a manner of seeming reaction to ant gene expression changes as well as seeming manipulation of ant gene expression.

All in all we can find clear signs of manipulation in this system as well as seeming side effects of infection. For example, the cuticle colouration and sclerotization and the immune response are highly connected in the datasets and we can find arguments against the possible fitness benefits for the parasite in this case. Another possible side effect is oxidative stress response that might not even be entirely visible in the ant's transcriptome as the cestode seems to minimize the oxidative stress in its host in order to improve its own functioning.

Zusammenfassung

Parasiten haben einen tiefgreifenden Einfluß auf ihre Wirte und sind können in der Lage, deren Phänotyp zu manipulieren, um die Chancen einer Übertragung auf den Endwirt zu erhöhen. Dies scheint auch bei dem Zestoden *Anomotaenia brevis* und seinem Zwischenwirt, der Ameise *Temnothorax nylanderi*, der Fall zu sein. Bei einer Infektion beobachten wir Veränderungen der Farbe und Sklerotisation der Kutikel, der Aktivität, der Lebensdauer, der Interaktionen mit Nestgenossen und der Muskelmasse. Diese Veränderungen gehen mit Veränderungen der Genexpression in der Ameise einher, so dass es schwierig ist festzustellen, ob der Zestode wirklich die Fäden in dieser Interaktion zieht oder ob die phänotypischen Veränderungen einfach nur Nebeneffekte der Zestodeninfektion sind. In dieser Dissertation habe ich verschiedene Methoden angewandt, um zu untersuchen, ob der Parasit wirklich die Fäden in dieser Interaktion zieht oder ob wir alternative Erklärungen für die Unterschiede in der Genexpression finden können.

Im ersten Kapitel haben wir die Auswirkungen der Parasitenbelastung auf dieses System untersucht. Wir verglichen die Genexpression von nicht infizierten, gering infizierten und stark infizierten Wirten sowie von Parasiten, die den Wirt mit wenigen anderen und vielen anderen koinfizieren. Wir beobachteten ähnliche Veränderungen bei Parasit und Wirt, wo beide unter höherem Stress litten und der Wirt sich des Parasiten stärker bewusst zu sein scheint, da der Parasit ein größeres Bedürfnis hat, sich der Immunantwort des Wirts zu entziehen.

In Kapitel 2 beschlossen wir, Ameisen mit bakteriellem Lipopolysaccharid zu injizieren, um den Unterschied zwischen der Immunreaktion auf den Zestoden und der Immunreaktion auf Bakterien auf Ebene der Genexpression zu verstehen. Wir kombinierten dies mit der Untersuchung des Darmmikrobioms, um zu sehen, welche Auswirkungen beide Immunreaktionen (auf den Parasiten und auf das Bakterium) auf den Wirt haben. Wir fanden eine große Menge an unterschiedlich exprimierten Genen, die auch die Immunantwort stark beeinflussen. Bei näherer Betrachtung stellten wir insbesondere fest, dass die Gene, die sowohl bei einer Parasiteninfektion als auch bei einer Scheininfektion unterschiedlich exprimiert werden, in verschiedene Richtungen unterschiedlich exprimiert werden. Dies deutet auf eine mögliche Hemmung des Immunsystems in diesem System hin. Diese Hemmung wirkte sich jedoch nicht auf das Immunsystem gegen Bakterien aus, was bedeutet, dass der Zestode in der Lage ist, die Angriffe gegen ihn zu minimieren, ohne die Fähigkeit der Ameise zur Bekämpfung anderer Parasiten zu beeinträchtigen. Im Darmmikrobiom fanden wir bei der Parasiteninfektion einen Verlust der Artenvielfalt und eine Dominanz intrazellulärer Gruppen in den infizierten Ameisen, was möglicherweise auf eine frühere Immunreaktion auf die Zestodeninfektion hindeutet.

Im letzten Kapitel testeten wir, wie sich die Prävalenz von Parasiten in diesem System auf die Genexpression der Kolonie auswirkt, indem wir die Genexpression mit dem Anteil infizierter Ameisen in der Kolonie sowohl bei infizierten Wirten als auch bei nicht infizierten Wirten und Parasiten korrelierten. Dabei stellten wir eine Stressreaktion bei den Ameisen fest. Während der Parasit, obwohl er in seiner Genexpression betroffen ist, unter diesen Bedingungen nicht zu leiden scheint. Darüber hinaus haben wir eine neuartige Methode zur Untersuchung des Interaktoms zwischen Parasit und Wirt angewandt, mit der wir ein neues Licht auf die molekularen Interaktionen geworfen haben. Dabei haben wir festgestellt, dass das Transkriptom des Parasiten in hohem Maße mit dem der Ameise interagiert,

und zwar sowohl in Form einer Reaktion auf Veränderungen der Genexpression der Ameise als auch in Form einer Manipulation der Genexpression der Ameise.

Insgesamt lassen sich in diesem System deutliche Anzeichen für Manipulationen und möglichen Nebenwirkungen der Infektion finden. Beispielsweise sind die Färbung und Sklerotisierung der Kutikula und die Immunreaktion in den Datensätzen eng miteinander verbunden, und wir können in diesem Fall Argumente vor und gegen einen möglichen Fitnessvorteil für den Parasiten finden. Ein weiterer möglicher Nebeneffekt ist die Reaktion auf oxidativen Stress, der im Transkriptom der Ameise nicht vollständig sichtbar ist, da der Zestode den oxidativen Stress in seinem Wirt zu minimieren scheint, um seine eigene Überlebensfähigkeit zu verbessern.

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General introduction

Parasites and their impact on the world

Parasites have always been all around us. Perhaps this is best illustrated through the fact that we have been finding parasites whenever we learned how to look for them. As early as the 15th century BCE we find descriptions of both tapeworms and roundworms in the ancient Egyptian Ebers papyrus (Ebbell, 1937). These descriptions became more detailed with Arabic physicians like Razi and Avicenna in the 10th and 11th centuries AD (Al-Razi, 10th century; Avicenna, 11th century). Later in the 17th century with the advent of the microscope, Antonie van Leeuwenhoek found the microscopic protist *Giardia duodenalis* in his own stool (Dobell, 1932). At the same time we also encounter the ”father” of modern parasitology, Francesco Redi, who described both ecto- and endoparasites on humans and other animals (Redi, 1668). With parasites being so prevalent, it’s no surprise that parasites have been hugely consequential to human history and indeed, the history of life on earth itself. Malaria for example has been recognized as the cause of population decline in several Ancient Greek city states (Pappas et al. 2008) and schistosomiasis was so common in Napoleon’s men in Egypt that it had been described as “the only country where men menstruate” (Sarant, 2017). As for the evolutionary history of parasites we find evidence of parasitism as far back as the early Cambrian period (Zhang et al. 2020). Meanwhile, parasitism has grown to be an evolutionary successful strategy with between 50% and 70% of species on earth being parasitic (Windsor, 1998). Parasitism of metazoans on other metazoans has evolved independently at least 60 times (Poulin & Morand, 2000). Because of this, parasites have often been an invisible influence on ecosystems worldwide. In coral reefs, for example, crustacean parasites have been found to be a natural means of population level regulation in reef fish (Forrester & Finley, 2006). When measuring the biomass of trematodes in a freshwater ecosystem, Preston and colleagues found that larval trematodes alone account for 25% of host and parasite biomass (Preston et al. 2013). Biomass is an important tool in understanding the flow of energy within an ecosystem (Odum & Barrett, 1971) and therefore parasites have unsurprisingly been shown to heavily affect the flow of nutrients in ecosystems (Kuris et al. 2008). It could also be that the absence of a parasite could create large advantages for a host species within an ecosystem. This is a reasonable assumption within the enemy release hypothesis (Keane & Crawley, 2002) which states that a lack of natural enemies (predators, parasites or otherwise) will result in a rapid increase of distribution and abundance of an invasive species (Morais & Reichard, 2018). Absence of parasites can therefore explain the success of invasive species and could be consequential for conservation efforts. With that information in mind, we could easily conclude that a world without parasites might not necessarily be a better one (Wood & Johnson, 2015).

This does not just count for parasites within an ecosystem but might even be the case for humans. While diseases like malaria continue to cause endless amounts of human suffering especially in the developing world with hundreds of thousands of deaths each year (WHO, 2022), the absence of parasites in humans have often been linked to auto-immune diseases in the so-called hygiene hypothesis (Bach, 2018; Okada et al. 2010; Stiemsma et al. 2015). This theory states that, in the industrialized world, the immune system does not learn to react to relatively mild disease agents causing the immune system to overreact to any threat thrown its way, causing eczema, allergies and general auto-immune diseases. Exposure to parasites, especially helminths seems to have positive effects for people suffering from autoimmune disorders and patients have even been subjected to helminth therapy (reviewed in Elliott & Weinstock, 2012; Helmby, 2015).

Parasite-host co-evolution

The fact that entire ecosystems are dependent on parasites and that humans, but also other organisms tend to poorly react to an absence of parasites provides a small window into parasite-host co-evolution. We can expend this window by talking about the effects of parasites on host fitness. In general, parasitism can be described as an association between two different organisms wherein one benefits at the expense of the other (Olano et al. 2011), without causing the death of the host. This negative fitness effect on their host of course might have a positive fitness effect on competitors in the same ecosystem. It would be somewhat comparable to the positive biodiversity effect that predators cause in ecosystems as shown by Robert Paine (1966) in his starfish removal experiment. Similar things have been demonstrated for parasites as well, most notably by Price and colleagues in 1986. They showed that parasites can regulate biodiversity by altering the competitive outcome between host species - a phenomenon called parasite-mediated competition (explained in figure 1a). Removal of parasites from ecosystems therefore might create a competitive advantage to species or individuals that are poorly adapted to parasitism, due to the significantly higher fitness cost of these species or individuals. The degree to which parasites and hosts are adapted to each other can not be understated. This is perhaps most easily exemplified by the existence of sickle cell anaemia in humans and its frequency in malaria-prone areas. Human sickle cell anaemia is a somewhat rare and highly lethal medical condition, which is caused by a single point mutation in the β-globin gene (Stamatoyannopoulos, 1972). When homozygous, carriers will often die prematurely due to sickle cell disease or other diseases, which pose a higher risk due to sickle cell disease (Lanzkron et al. 2013). For this reason, it is rare in most of the world due to it being selected out of the gene pool. The major exception is West Africa where over 80% of global sickle cell anaemia cases occur (Piel et al. 2013). This has to do with the fact that the sickle cell allele, even in heterozygotes, increases ones resistance to malaria (Ackerman et al. 2005; Hill et al. 1991). Therefore, the heterozygous fitness advantages in malaria-prone areas still allow for the existence of lethal homozygotes (figure 1b). Somewhat tragically however, it seems that malaria in turn has found a way to evade sickle cell anaemia and some *Plasmodium falciparum* genotypes have been correlated to severe malaria even in presumed protected individuals (Band et al. 2022).

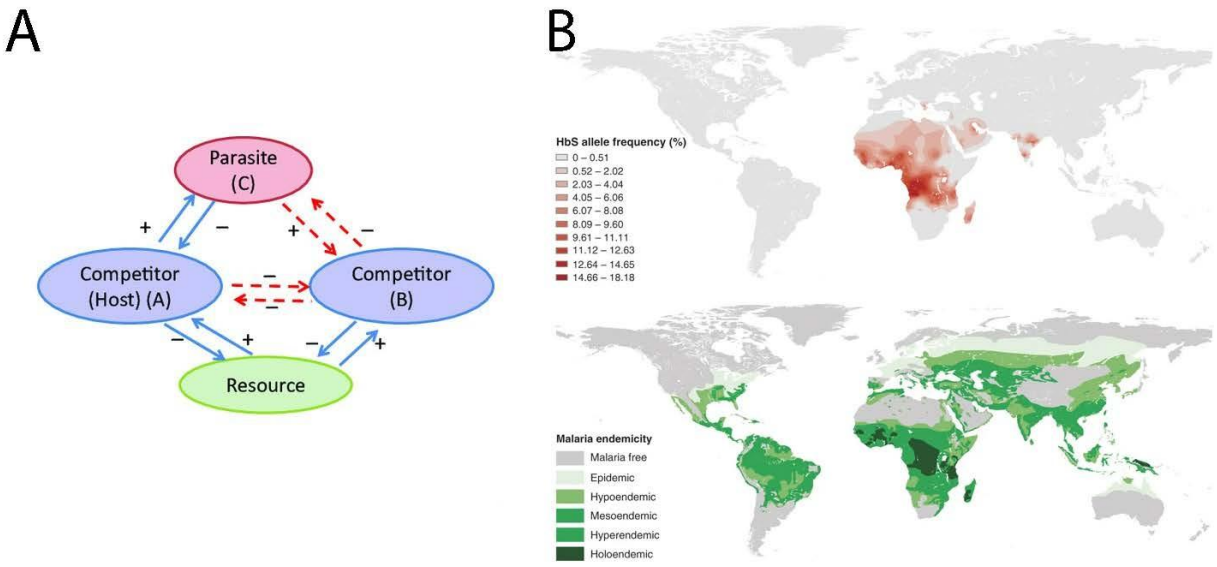

Figure 1 A. Example of a model with parasite mediated competition where the presence of a parasite can have a potential positive impact on species B, extracted from (Dunn et al. 2012). **B.** The allele frequency of the HbS allele causing sickle cell anaemia (up) and the endemicity of malaria (down) illustrated on a world map, extracted from (Piel et al. 2010).

This also shows that, from the parasite's perspective, the effect of its presence of the host is also to consider. Many parasites would prefer to remain in their host for a very long time but at the same time, prefer to take as many resources from their host as possible. This can be paradoxical since higher host exploitation will lead to a shorter lifespan of the host (Frank, 1996). However, parasites are often at least capable of targeting specific parts of the host that do not affect the host's survival. For example, parasites commonly target the host's reproductive system (Kaltungo & Musa, 2013), which, though essential for host fitness, does not necessarily influence the host's survival. The same can be seen in the fact that infectious diseases often target the host thymus (Savino, 2006), whose absence does not result in the immediate death of the host as the liver, heart, brain or lungs would. Another piece of evidence that parasites try not to be detrimental to the host can be shown through the effects of unwanted cross-species transmission. Whenever a parasite adapted to a specific host is capable of infecting another host, the parasite is much more deadly in this novel host. The best examples can be found in human history where humans, living close to farm animals, have the tendency to contract relatively harmless diseases from these animals with disastrous consequences. Ebola for example, is a relatively harmless virus in fruit bats, which are able to live with the virus for a long time (Hayman et al. 2010). In humans however, the virus is highly lethal with an up to 90% fatality rate. An even more recent example of a highly deadly disease is with COVID-19, which shows close genetic similarity with a coronavirus originating from bats (Latinne et al. 2020). However, there are also parasites that are adapted quite thoroughly to multiple host and even infect multiple species within their lifespan. These are generally known as parasites with a complex life cycle.

Parasites with complex life cycles

Some parasites have multiple obligatory hosts, this means that without infecting several hosts in succession, the parasite cannot complete its life cycle. Usually, these parasites switch hosts through either upward or downward incorporation, meaning the movement upward or downward on a scale of trophic levels respectively. Upward incorporation is usually trophic transmission where an intermediate host is being preyed upon by a definitive host, whereas downward incorporation typically necessitates a free-living stage or a mechanism through which intermediate hosts ingest the parasite transmission stages (Parker et al. 2003). A well-studied example is the case with the cestode *Schistocephalus solidus*, whose life cycle necessitates a free living stage to infect copepods, these copepods need to be eaten by sticklebacks and the sticklebacks need to be eaten by birds, which need to defecate in the water (Clarke, 1954). Due to the complexity of these life cycles, one might expect that these types of parasites are relatively rare, but parasites are nothing if not paradoxical and there have been cases that over 75% of all parasites encountered in an ecosystem infected multiple hosts (Wood et al. 2023). Due to these parasites being transmitted through host-host interactions, the presence of parasites with complex life cycles has in fact even been used as indicator of a healthy ecosystem (Hudson et al. 2006). Not surprisingly, these parasites are also hit the hardest when the ecosystem is in a worse state and these multi-host parasites have suffered greatly from anthropogenic climate change (Wood et al. 2023). The reason that these parasites are often still very common however is because parasites with complex life cycles can profit from the best of both worlds. Larger hosts infected through upward incorporation typically allow for a longer lifespan, greater body size and an increased fecundity, whereas smaller hosts infected through downward incorporation have the potential to increase transmission (Auld & Tinsley, 2015; Parker et al. 2003). Another advantage of upward incorporation is that predators are always in lower numbers than prey species, meaning that a predator ingesting multiple infected individuals could

act as “concentrators” of parasites, making it easier to find a mate for the parasites, increasing genetic diversity (Brown et al. 2001).

The disadvantages of a complex life cycle are mainly twofold. Firstly, with multiple hosts, a parasite needs to adapt to both hosts. The morphological, chemical and physiological differences between hosts can be extreme. For example, trematodes typically have a vertebrate and a gastropod as a host (Cribb et al. 2003; Schmid-hempel, 2008), whereas cestodes can switch between anything from multiple vertebrates or vertebrates and arthropods (Mackiewicz, 1988). This host switching over different superphyla would necessitate enormous adaptations to immune systems that have evolved completely different mechanisms to fight pathogenic threats (Yuan et al. 2014). It seems one way to adapt to both host’s immune defences is by adapting to the commonalities in both species, considering previous research on this topic found that cestodes that did well in their invertebrate intermediate host were also able to avoid innate immune detection with their vertebrate final host (Hammerschmidt & Kurtz, 2005). The second disadvantage of a complex life cycle, at least in the case of parasites that are trophically transmitted, is that they are dependent on intermediate and final hosts encountering one another. This is of course especially at odds with a host that needs to be consumed for the parasite to complete its life cycle. In order to achieve this it seems that parasites have often evolved the ability to manipulate their host into increasing the chances of encountering the final host. Most famously this is the case with *Toxoplasma gondii* which, when infecting mice, cause the mice to completely lose their fear of cats, sometimes even seeking them out (Ingram et al. 2013). This host manipulation has become its own subfield in evolutionary parasitology which focusses on how and if parasites are capable of manipulating the host.

Host manipulation by parasites

The idea that parasites are capable of manipulating the host’s phenotype was conceptualized by Bethel and Holmes in the 1970s (Bethel & Holmes, 1973) and gained popularity with Richard Dawkins’ book “The Extended Phenotype” (Dawkins, 1982). Here he states that the phenotypes of organisms are not only limited to biological processes that influence the individual, but rather the influence a gene can have on the environment outside of the organism. This is manifested in, for example, the environment with beaver dams but can also extend to other organisms such as the manipulation of host phenotypes by parasites. Aside from the example mentioned in the previous section of *Toxoplasma gondii*, countless phenotype changes upon parasite infection have been proposed as examples of parasite-mediated manipulation. Recently it was discovered that female mosquitoes carrying the malaria parasite *Plasmodium falciparum* were more attracted to human breath and odours than mosquitoes that did not carry malaria (Vantaux et al. 2021). An example that especially caught the public imagination is that of the fungus *Ophiocordyceps unilateralis* which is capable of manipulating the behaviour of ants, which, when infected, climb to the tops of trees where they attach themselves to the surface of leaves with their mandibles where they die (figure 2a). The fungus can then spread its spores more easily from this higher position (Evans et al. 2011; Weinersmith & Faulkes, 2014). As mentioned earlier, an important question to this field of parasitology is how the parasite is capable of causing such changes to the phenotype. In some cases, this is partially resolved, the most obvious example being in gordian worms. These worms induce suicidal behaviour in their intermediate cricket host causing them to jump into the water in which the worms can complete their life cycle (figure 2b). One reason why infected crickets might do that is because the parasite modulates the cricket’s response to light, specifically to light coming from below which can be reflected on the water (Ponton et al. 2011).

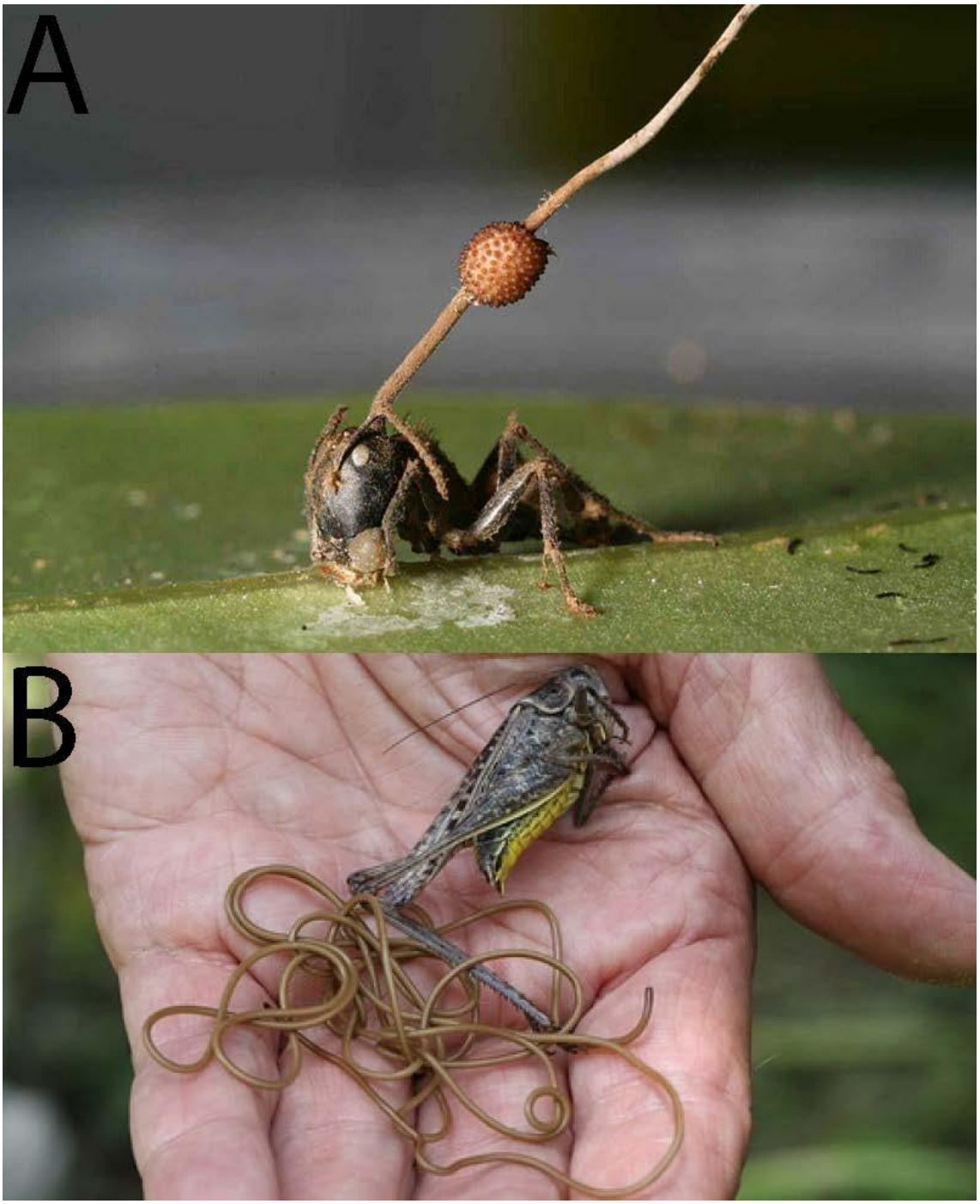

Figure 2 Examples of parasites that manipulate their hosts neurologically **A.** *Ophiocordyceps unilateralis* growing out of a dead ant having walked to the top of a tree (photo: D. Hughes). and **B.** Gordian worms crawling out of a dead cricket after causing it to jump to its death (photo: Alastair Rae).

However, within the field of evolutionary parasitology phenotypic changes like these were often discussed as adaptive to the parasite and specifically parasite-induced without providing hard evidence (Cézilly et al. 2010; Poulin & Maure, 2015). This changed around the turn of the millennium where people started arguing that, although these changes might provide fitness benefits for the parasite, they could be mere by-products of parasite infection or the changes caused by a parasite in the host's immune system (Poulin et al. 1994).

Phenotype changes as a side effect

Although there are some parasite-induced phenotypic changes that are undoubtedly the product of parasite manipulation, there are definitely cases where the line between manipulation and neutral side effect is blurred. The part of the host that interacts the most with the parasite is of course the immune system, which has often been shown to be actively manipulated by the parasite. For example, *Toxoplasma* is capable of affecting interferon regulation by secreting proteins that activate host kinases (Brumlik et al. 2013). In contrast, the behavioural changes that *Toxoplasma* induces in humans have been prescribed as not necessarily adaptive to the parasite but correlative to the adverse health effects of toxoplasmosis (Flegr et al. 2023). Often these stories get even more complicated through the interference of interactive microbiota. The nematode *Trichuris muris* for example has shown to cause changes to the mouse host microbiome that prevents other parasites from infecting the host but also facilitate long term infection for the nematode itself (White et al. 2018). However, although these changes to the microbiome themselves might be advantageous to the parasite, other phenotypic modulations that this specific microbiome may cause can then be considered a side effect. This while it has been long known that microbiota can have profound effects on their host's phenotype (reviewed by Lynch & Hsiao, 2019). Furthermore, the host itself is also perfectly capable of changing its own phenotype and parasite infection could serve as a means of breaking down a canalized phenotype due to infection stress (Jojić et al. 2021). Simply comparing the phenotype of a host with an uninfected individual of the same species therefore might not be enough to determine whether a parasite is the one pulling the strings even though the phenotypic changes to the host are entirely unique with parasite infection. Several characteristics of the infected host prevent one from doing that, the gut microbiome could be different, the immune reaction to the parasite might create an energetic trade-off that impairs the development of certain phenotypic characteristics (Hicks et al. 2018) or the parasite's actual manipulation mechanisms create unwanted side effects. Therefore, when considering a parasite-induced phenotype, one has to investigate not only the interaction between parasite and host, but also the interaction of other species with either parasite or host. One also has to regard the entire phenotypic change to the host to find out which characteristics are most likely to be caused by the parasite directly and which ones are mere side-effects. And finally, one has to take into account the host's potential for phenotypic plasticity.

Parasitism in social insects

The kings, or rather the queens of phenotypic plasticity might be eusocial insects, these are mainly represented by social Hymenoptera such as ants, bees and paper wasps as well as by termites and to a lower degree some species of Hemiptera and Thysanoptera. These systems are especially characterized by their organization of reproductive and non-reproductive castes between highly related individuals of multiple generations (Wilson, 1971). These different castes display large phenotypic diversity, which is especially visible in ants (Hymenoptera: Formicidae). Here the reproductive queens are usually the longest-lived, only fertile and largest individual whereas workers specialize in specific tasks such as brood care (nurses), foraging (foragers) and defence (in some species, soldiers). This eusocial lifestyle has elevated ants alone as a hugely successful family of insects with an estimated global biomass of 12 megatons, which exceeds that of all wild birds and mammals combined (Schultheiss et al. 2022). Key to this social lifestyle is communication through chemical signatures, which ants use to recognize queens, other nestmates and ants from different colonies (Ferguson et al. 2020). Through specific chemicals on the cuticle worker ants know which nestmates to feed and which to ignore.

This system of course is prone to exploitation by parasites and there are several parasites capable of mimicking this cuticular hydrocarbon profile. What serves as an obvious example are butterflies of the genus *Phengaris*, whose larvae crawl into ant nests and can mimic the hydrocarbon profile of the ant brood so that workers treat them like larvae from their own colony (Akino et al. 1999). This social parasitism is also seen in dulotic ant species which infiltrate colonies of another species in order to either steal brood and have these ants work for them or kill the host queen to have the remaining workers raise their brood along with other strategies (Helantera, 2021). These parasitic ants also have very precise molecular mimicry in order to infiltrate host colonies (Johnson et al. 2005). Ant societies are also plagued by a large number of endoparasites that seem to exploit the phenotypic plasticity of ants. For example, multiple nematodes from the family of Mermithidae use ant species as intermediate hosts (Poinar, 2012). When infected, ants develop a sort of caste mosaic, displaying characteristics from foragers, queens, soldiers and nurses as well as entirely unique phenotypic characteristics to infected ants (Laciny et al. 2017). When regarding other families of nematodes, ant parasites can also be found in Tetradonematidae, specifically the species *Tetradonema solenopsis* which parasitizes on the fire ant *Solenopsis invicta*, which develop an enlarged abdomen upon infection (Briano et al. 2012). Cestodes are also known to cause profound phenotypic changes to ants when they act as an intermediate host. The Davaineid cestode *Raillietina urogalli*, which infects turkeys as a final host, is known to use *Myrmica* species as an intermediate host (W. M. Reid & Nugara, 1961). The most commonly observed phenotypic change here is a dark-reddish colouration of the cuticle (Muir, 1954). This colour difference may be due to the formation of a melanoid pigment from the excretions of the parasite (Laciny, 2021; Muir, 1954).

Temnothorax nylanderi and *Anomotaenia brevis*

This finally leads us to the system that is central to this doctoral thesis, the ant *Temnothorax nylanderi* (figure 3a) and the cestode *Anomotaenia brevis* (figure 3b) from the family of Dilepididae. Infected ants show perhaps among the most extreme and diverse phenotypic changes upon parasite infection mentioned in this text. These changes include yellow colouration of the cuticle (Plateaux, 1972; shown in figure 3a), sluggish behaviour (Trabalon et al. 2000) with underdeveloped muscles (Feldmeyer et al. 2016), a smaller body size (Scharf et al. 2012) and a far longer lifespan than uninfected nestmates (Beros et al. 2021). Aside from these changes, infected ants seem to also have an effect on their uninfected nestmates, which socially care for them more often (Beros et al. 2019) and they live shorter than in uninfected nests (Beros et al. 2021). These changes are believed to aid *A. brevis* in the completion of its life cycle, as infected ants, the intermediate host, have a higher chance of being eaten by woodpeckers, the final host figure 3c). Where, for example, the longer lifespan increases the chance of woodpeckers encountering the nest with the infected ants still alive and the sluggish behaviour making them easier to catch. Another hypothesis regarding this system is that *A. brevis* infection achieves such phenotypic changes because of changes that it causes in the ant's gene expression. As mentioned earlier, the incredible amount of phenotypic plasticity these ants display can therefore be prone to parasite manipulation. The reduced pigmentation and sclerotization of the cuticle is a clear example of maldevelopment of the cuticle and the long lifespan is similar to that of queens (Beros et al. 2021) which means the potential for a long lifespan rests in the genome of all these ants. Indeed, gene expression differences have been observed in this species upon parasite infection, among which genes involved in muscle development (Feldmeyer et al. 2016) and anti-ageing (Stoldt et al. 2021). This coincides with the excretion of proteins of the cestode in the ant's haemolymph (Hartke et al. 2023), which, among others, have antioxidant functions. Taking this system as a whole with the information given in this text, there

is a large potential of investigating these parasite-induced phenotypic changes in *T. nylanderi*. At surface level, the parasite-induced phenotypic changes seem to benefit the parasite's fitness, and the ant's phenotypic plasticity is ripe for manipulation. On the other hand there is a lot of interference within this system. Due to the large number of phenotypic changes, who is to say which characteristics are just a side effect of something *A. brevis* actually actively manipulates? What is the role of other species in this? But also, how does this "extended phenotype" reflect upon the colony, as in a social system, the interaction between nestmates is extremely important for an insect's phenotype (Smith et al. 2008; Watanabe et al. 2014).

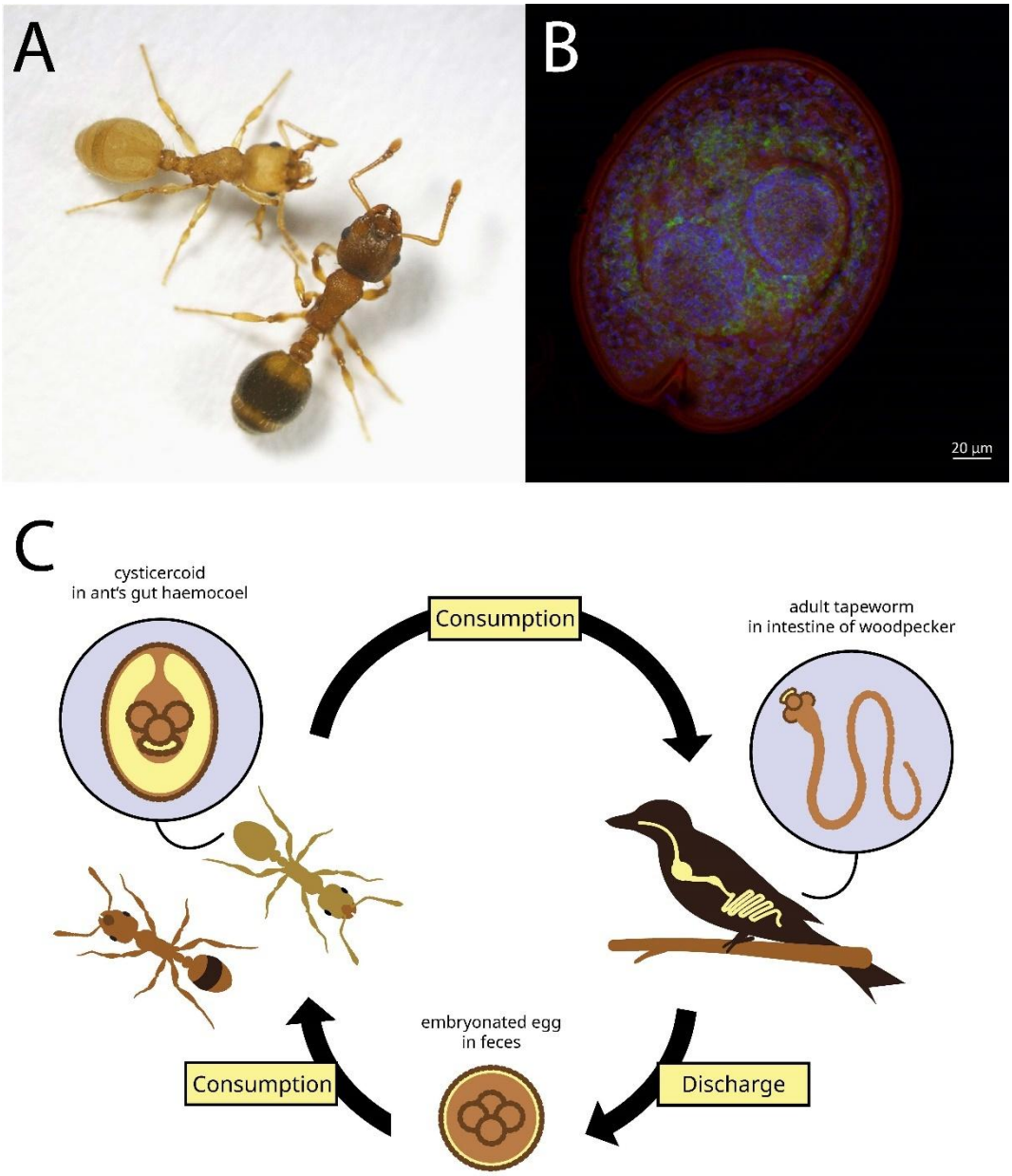

Figure 3 A. Two individuals of *Temnothorax nylanderi* one of which (upper left) is infected with *Anomotaenia brevis* (photo: Susanne Foitzik). **B.** Fluorescence in situ hybridization picture of *A. brevis* (photo: Benjamin Weiss). **C.** The life cycle of *A. brevis* visualized, illustrated for Sistermans et al. 2023 by Jenny Fuchs.

Aims of this doctoral thesis

The question whether *A. brevis* manipulates the phenotype of *T. nylanderi* is central to this thesis. In the introduction we've seen several ways in which the phenotype of a parasitized individual can be influenced, directly or indirectly, by said infection, many of these possibilities have not been tested. This helps us shed light on the co-evolution of *A. brevis* and *T. nylanderi*, which traits are more likely to be adaptive for the parasite, which traits are more likely to be side effects of infection and which traits are possibly caused by other interfering factors. This is done through three different experiments that all explore the dynamics of gene expression in both parasite and host. In the first chapter we explore whether parasite load, or the number of parasites per host affects the gene expression of both parasite and host. Through this method we can identify characteristics that become more easy or harder to manipulate for the parasite depending on how many there are in the host. On one hand, higher parasite load should make the manipulation easier, as more cestodes can divert less resources to host manipulation. On the other hand, there are also less resources available per cestode. This also counts for the host, which should bear a higher cost of parasitism with a high parasite load. In the second chapter we then place one specific characteristic, the immune reaction, in the spotlight. By immune challenging the host with a mock bacterial threat, we can identify specific immune pathways of the host towards the cestode and towards bacteria. It is also possible to identify common innate immune pathways and their directionality through gene expression and whether the parasite is capable of inhibiting certain immune pathways. Additionally we investigated these factors' influence on the gut microbiome, which, as we mentioned earlier, could also influence certain phenotypic characteristics of the ant, whether advantageous for the cestode or not. Finally, in the third chapter, we investigate the effect of the cestode on a superorganism level. The higher the prevalence of infected workers in the colony, the uninfected ants bearing the brunt of their nestmates' infection might also be more strongly affected. Never mind the fact that infected ants now have to compete among each other for the more limited resource of nestmate care, in possibly a similar manner to cestodes in the first chapter. As we took cestodes and ants from the same individuals for this experiment, we also added a final analysis to this chapter where we identify the interactome of parasite and host. We tested whether certain parasite genes were more likely to co-express with host genes, identified these interaction partners and discuss the possible consequences to the host phenotype. All in all, I believe the different ways to shed light on the gene-gene interactions between parasite and host allows us to see how millions of years of co-evolution gave the parasite an ability to understand their host's genotype-phenotype map to a degree that is still largely unknown by biological research in general. And I would argue that, as uncomfortable as parasites may make many not in the field, they have a lot to teach us about the fundamental processes of life on earth.

Chapter 1

The influence of parasite load on transcriptional activity and morphology of a cestode and its ant intermediate host

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Abstract

Parasites with complex life cycles are known to induce phenotypic changes in their intermediate hosts to increase transmission to the final host. The magnitude of these changes could increase with the number of parasites, which would be beneficial to co-infecting parasites. Yet, adverse effects of high parasite load (i.e., many parasites in a single host) might stress both hosts and parasites (e.g., through an increased immune response). We investigated the consequences of parasite load on the transcriptional activity and morphology of the cestode *Anomotaenia brevis* and its intermediate host, the ant *Temnothorax nylanderi*. We demonstrated that many differentially expressed host genes shifted with parasite load, and their functions indicate a stronger immune response and fight against oxidative stress in heavily infected hosts. The expression of other host genes responded to infection in an all-or-nothing manner, as did the morphology of the host workers. However, the cestodes became smaller when they competed with other parasites for resources from a single host. Their expression profile further indicated shifts in host immune avoidance, starvation resistance and vesicle-mediated transport. In summary, our study reveals clear consequences of parasite load and highlights specific processes and traits affected by this.

Keywords: host-parasite interaction, parasite burden, manipulation, parasite escape, cestode

Introduction

Parasite infection often leads to phenotypic changes in the host that appear to benefit the parasite (Poulin & Thomas, 1999). These shifts in morphology, physiology, behaviour or other life history traits are particularly pronounced and well-studied in parasites with complex life cycles that exploit multiple host species (Heil, 2016). Examples include the induction of fruit mimicry in ants by the nematode *Myrmeconema neotropicum* (Yanoviak et al. 2008) and the reduction of predator aversion in rodents as a result of *Toxoplasma gondii* infection (Dass & Vyas, 2014). In general, these phenotypic changes are thought to facilitate transfer from intermediate to definitive hosts (Poulin & Thomas, 1999; Weinersmith & Faulkes, 2014).

A common interpretation in evolutionary parasitology is to consider phenotypic changes in infected hosts as the "extended phenotype" of the parasite, when they are encoded in the parasite's genome and consequently are subject to selection in the parasite (Dawkins, 1982; Moore, 2002). This view has been criticized in recent years due to a lack of experimental evidence on the consequences of these phenotypic shifts for the fitness of the parasite (Cézilly et al. 2013; 2010; Poulin & Maure, 2015). The change in host phenotype that follows infection may also be a by-product of infection that is not beneficial to either party, or an adaptive compensatory response of the host to limit the negative consequences of parasite exploitation (Lefèvre et al. 2008; Minchella, 1985; Poulin, et al. 1994).

One way to gain deeper insights into the causes of the altered host phenotype upon infection is to study the consequences of parasite load on the phenotype and fitness of both interacting partners. We might detect divergent phenotypic changes in the host depending on who is responsible for said changes (parasite or host). For example, Maksimowich and Mathis (2000) explored aggression of the salamander *Plethodon ouachitae* in response to high parasite load of the chigger mite *Hannemania dunni*. They found that highly infected hosts were less aggressive than lowly infected hosts even though higher aggression was found to be advantageous for the parasite, aiding in its transmission (Maksimowich & Mathis, 2000). In another example, haemoparasite load affected the throat colour of male Iberian lizards (Megía-Palma et al. 2018), informing females about a male`s ability to deal with parasitic infections.

Another aspect that can be investigated through the lens of parasite load is the consequences for the parasite, for which an increased infection intensity can have diverse outcomes. Benefits for the parasite include synergistic effects, as individual parasites need to invest less to manipulate the host or alter its phenotype to facilitate transmission to the final host (Franceschi, et al. 2008; Yan et al. 2018). This could lead to stronger fitness benefits for the parasite, for example in the acanthocephalan parasite *Pomphorhynchus laevis*, which causes its host to leave its hiding places more often under higher parasite load, aiding in transmission to new hosts (Labaude et al. 2020). Negative consequences are more competition among parasites, as multiple individuals need to share their host's resources (Bush & Lotz, 2000). Furthermore, a higher parasite load could trigger stronger host immune defences that in turn could harm the parasites (Kaunisto & Suhonen, 2013). Finally, high parasite load might cause premature host death, consequently reducing parasite fitness (De Roode et al. 2008). In some systems, these trade-offs could also affect the phenotype of the parasite, which is particularly well-studied in cestodes, where crowding results in the development of fewer proglottids, i. e. reproductive segments (Heins et al. 2002; Holmes, 1961).

To investigate the effects of parasite load, we used the cestode *Anomotaenia brevis* and its intermediate host, the ant *Temnothorax nylanderi* as a model system. Adult individuals of this tapeworm species reproduce sexually in the gut of birds, most commonly woodpeckers (Plateaux, 1972; Trabalon et al. 2000). Their eggs are released in bird faeces, which *T. nylanderi* ants use to feed their larvae and thus infecting them with *A. brevis*. The parasite larvae penetrate the ant's gut and develop into cysticercoids in the haemolymph. They remain in this larval stage until the ants are consumed by avian predators (Fig. S1). The phenotype of *T. nylanderi* workers is strongly altered following infection. Their cuticle is less sclerotized and pigmented during the pupal stage, leading to adult ants exhibiting a lighter, softer cuticle (Plateaux, 1972). Moreover, infected workers are less active, suffer from muscular dystrophy (Feldmeyer et al. 2016), receive more social attention (Scharf et al. 2012) and live substantially longer than their uninfected nestmates (Beros et al. 2021). A three-year study (Beros et al. 2021) found no difference in survival between infected workers and uninfected queens; the latter can live up to two decades in this species. Many of the physiological changes can be interpreted as being advantageous to the cestode and go together with transcriptomic shifts that might explain some of the altered traits of infected workers (Beros et al. 2021; Stoldt et al. 2021; Trabalon et al. 2000). For example, genes related to muscle development are downregulated in infected workers (Feldmeyer et al. 2016), possibly explaining not only muscle maldevelopment, but also the reduced ability of infected workers to escape when a nest is opened by a predator (Beros et al. 2015). Upregulation of genes related to oxidative stress resistance (Stoldt et al. 2021) might explain their extended lifespan, which in turn could increase the likelihood that infected workers will be preyed upon by the parasite's final host, the woodpecker.

The consequences of infection by multiple parasite individuals have not been studied in this system, despite a high variance in parasite load. While some ant workers are infected by a single cestode, others can harbour more than 70 cysticercoids in their haemolymph (data from this study, see below). Here, we exploit this variance to gain deeper insights into the complex relationship between *A. brevis* and *T. nylanderi*. We study the consequences of parasite load on the transcriptional activity and morphology of *A. brevis* and *T. nylanderi*. We predict that a higher parasite load should lead to more stress in both host and parasite. For the host, a high parasite burden may lead to higher potential of the parasite to damage the host (De Roode et al. 2008), possibly resulting in smaller body size and greater expression of stress genes in heavily infected individuals. If the parasites are unable to evade host recognition, a high parasite load might also exhibit an enhanced immune response. However, in view of the possibility of host manipulation, a higher parasite load should also lead to more extreme phenotypic changes in the host, which might be to the advantage of the parasite. For example, many parasites together may be better at subverting the typical innate immune response of insects, which includes encapsulation and melanisation, or they may be better at upregulating host genes important for responding to oxidative stress to extend host lifespan. When looking at the parasite, crowding is a well-studied aspect of cestode biology (Heins et al. 2002), including early life stages (Benesh, 2019). Therefore, we predict cysticercoids to be smaller in heavily infected hosts. In terms of transcriptomic shifts, we expect genes involved in parasite-host communication, i.e. those involved in either phenotype manipulation or immune evasion of the host, to be differentially expressed. This should lead to a down-regulation of these functions at high parasite load, as individual cestodes would need to invest less in host communication. However, we hypothesise that genes involved in communication and competition with

other parasites are upregulated because competition between densely packed cestodes is likely to be greater. In addition, we predict an overexpression of genes that aid in nutrient acquisition or even in combating starvation, as parasites that must share their host with several competitors are likely to have fewer resources available to them.

Material and methods

Collection and sampling of ants

Colonies of *T. nylanderi* were collected in November 2019 and February 2020 in the Lenneberg Forest near Mainz, Germany (50.011605" N, 8°10'48.6" E). Colonies of this ant species, whose workers are only about 2-3 mm long, include several dozen workers and inhabit dead branches and acorns on the forest floor. From the colonies we found, we selected 25 colonies, 21 containing infected workers (Table S1). Infection was initially determined by the yellow colouration of infected workers, a sign of infection (Scharf et al. 2012). The ants were transferred to slide nests consisting of a Plexiglas cavity between two slides and placed in plastered nest boxes with three chambers (Stoldt et al. 2021). Colonies were maintained at 20+/-1°C and fed with small crickets and honey twice per week. Water was provided ad libitum. The number of infected workers and queens were counted, and colonies were kept under these conditions for six months.

At the end of July 2020, all infected workers, and a sample of several uninfected workers per colony were collected and dissected (N of dissected, uninfected workers per colony see Table S1). Furthermore, we dissected five workers per colony from four uninfected colonies. For all ant workers that were dissected, the presence and number of cysticercoid cestode larvae was noted and their diameter was measured. Furthermore, worker head width was measured from eye to eye, as host body size was shown to decrease in *T. nylanderi* (Scharf et al. 2012) and other systems with increasing parasite load (Dingemanse et al. 2009). All measurements were taken under a Leica stereomicroscope 200x magnification using the LAS v4.5 software.

So far, we cannot infect the ants artificially with the cestode and therefore had to resort to naturally infected ants from the field. Infected ants differ from healthy ones in many characteristics: they sclerotize and pigment their cuticle less during the pupal stage (Plateaux, 1972), they have a prolonged lifespan (Beros et al. 2021) and show altered behaviours, e.g., lower activity (Scharf et al. 2012). Whether these traits shift further with parasite load has not yet been investigated. Less than 1% of cestode species have asexually reproducing larvae (Mackiewicz, 1988), but among them are members of the family of Dilepididae (Gabrion & Helluy, 1982), to which *Anomotaenia brevis* belongs. However, we could not find any evidence in the literature that cysticercoid larvae of this species can asexually reproduce, nor did we ever observe asexual reproduction in the intermediate host ourselves. We therefore do not assume that heavily infected ants are older only because the parasites in older hosts had more time to multiply. We discuss our results below also in terms of whether possible age or activity differences could explain them.The fat body of the ants was extracted within five minutes of starting the dissection. We focused our transcriptome analysis on this tissue because it serves as a storage organ and plays an important role in the production of proteins for immune defence, fighting ageing, and fertility (Arrese & Soulages, 2010; Negroni, et al. 2019). We obtained the fat body by isolating the first

cuticular segment of the abdomen and removing the gut and trachea. What remains were fat cells and the cuticular segment itself, which was promptly transferred to Eppendorf tubes containing 50µl of Trizol, frozen on dry ice and stored at -80°C until RNA extraction. The cestodes were removed from around the midgut, where they are usually located in the haemolymph and separately transferred into Eppendorf tubes containing 50µl of Trizol. They were also frozen on dry ice and stored at -80°C until RNA extraction.

We selected seven infected colonies for transcriptomic analyses with infected workers with different parasite loads (Table S1). These colonies contained at least one queen and on average 93 workers (mean 93.1±43.4). Two of the infected colonies were collected in the field in November 2019, the other five in February 2020 (Table S1). Infected colonies contained between 2 and 24 infected workers and the infection rate varied between 3% and 22%. For each infected colony, we sampled the fat body of one highly infected worker (high parasite load; 18+ cysticercoids), one lowly infected worker (low parasite load; 1-6 cysticercoids) and an uninfected worker (Table S2). The threshold for high parasite load was set by including only ants infected with at least twice as many cestodes as the median parasite load in all samples (median=7). For low parasite load, we included the infected workers with the fewest parasites in their colony. For RNA extraction we homogenized the samples in Trizol using a plastic autoclaved micropistil. We then added 25µl of chloroform and centrifuged for 15 minutes. Afterwards the upper aqueous phase was transferred into Eppendorf tubes containing 25µl of 96% ethanol. RNA was isolated using the Qiagen RNeasy mini extraction kit following protocol specifications. Additionally, we extracted the RNA of one worker per uninfected colony (colony size: 95.8±44.8 workers). One of these colonies was taken from the field in November 2019 and the other three in February 2020. Sequencing was conducted by Novogene (Cambridge, UK). They enriched the mRNA using oligo(dT) beads, then fragmented randomly by adding fragmentation buffer. The cDNA was synthesized by using mRNA template and random hexamers primer, after which a custom second-strand synthesis buffer (Illumina), dNTPs, RNase H and DNA polymerase I were added to initiate the second-strand synthesis. After a series of terminal repair, A ligation and sequencing adaptor ligation, the cDNA libraries were completed through size selection and PCR enrichment. Illumina sequencing was conducted after pooling according to the libraries' effective concentration and expected data volume (Novogene Co., Ltd). We sequenced a minimum of 9Gb per sample, resulting in around 30 million paired-end 150bp reads.

We isolated the RNA of cysticercoids of five highly infected workers (between 18 and 51 cysticercoids, median = 23) and five lowly infected workers (four ants with one cysticercoid, one ant with two) from five colonies, which were also included in the host gene expression analyses (table S2.The RNA was isolated using the same protocol as the fat body samples and samples were sent to Novogene for RNA amplification using the SMARTer kit and RNA sequencing. None of our cestode samples had a lower RNA yield than 9ng, meaning we can assume that the amplification was reliable, as SMARTer amplification only becomes less reliable with an RNA yield below 1ng (Palomares et al. 2019). Library preparation and sequencing were done in the same way as described above. We sequenced a minimum of 9Gb per sample, resulting in around 30 million paired-end 150bp reads.

Morphometric analysis

To investigate the influence of cestode infection on host body size we conducted two comparisons. First, we analysed whether head width varied between uninfected workers (n = 100) and infected workers (n = 275) from infected colonies (n = 21 colonies) using a linear mixed effects model taking colony identity into account. Second, to investigate the influence of parasite load directly, we focused only on infected workers (n = 267) and used a linear mixed effects model, with colony identity as random effect (n = 21 colonies). For the analysis of parasite load on cestode body size, we again used a linear mixed effects model with colony and ant identity as random effects to investigate the impact of cestode number on cestode diameter, including 2980 cestodes belonging to 267 ants from 21 colonies. Due to the cestode data distribution being logarithmic, we log transformed the model during our analysis. All models were checked for normal distribution of their residuals and analysed using an ANOVA.

Differential gene expression analysis

We removed adapters and contaminating sequences from *A. brevis*, *Homo sapiens, Escherichia coli* and vectors from the ant reads using FastQScreen v0.14.0 (Wingett & Andrews, 2018). Then, the raw reads were trimmed with fastp (Chen et al. 2018) in paired-end mode for reads of 120 bp or more and an N-base cutoff of 15. Subsequently, the quality of the reads was assessed using FastQC (Andrews, 2010), which calculates the probability that a base call is erroneous. Filtered reads were mapped against the *T. nylanderi* genome assembly (Jongepier et al. 2022) using HISAT2 v2.1.0 (Kim et al. 2015) with the *--dta* parameter in preparation for genome-guided assembly. The resulting BAM files were sorted and indexed using Samtools v0.1.19 (Li et al. 2009) and used to generate a genome-guided transcriptome assembly using StringTie v1.3.6 (Pertea et al. 2015). To extract transcript sequences, gffread v0.11.4 was applied to the merged GTF files. To count gene abundances, we made use of the script prepDE.py (retrieved from StringTie webpage, Pertea et al. 2015). Subsequently, the predicted amino acid sequences of the transcripts were retrieved using TransDecoder v5.5.0 (Haas et al. 2013) and functionally annotated using InterProScan v5.46-81.0 (Jones et al. 2014). Finally, we obtained the KEGG IDs of the amino acid sequences using the online tool of EGGNOG mapper 2.1.8 (Cantalapiedra et al. 2021). KEGG pathways provide insights into the network of gene products.

The gene count matrix was analyzed using DESeq2 (Love et al. 2014), fitting a negative binomial distribution. We used a likelihood ratio test (LRT) to compare a model that included parasite load and colony identity as factors, to a reduced model including only colony identity. Gene expression was considered to differ between at least two of the parasite load categories when the Benjamini-Hochberg adjusted p-value was below 0.05 resulting in a list of ant differentially expressed genes (DEGs). Principal component analysis was performed to visualize whether parasite load caused clustering of samples. We ran a cluster analysis on the ant differentially expressed genes using DEGreport (Pantano, 2022) to identify groups of genes based on the similarity of their expression patterns. The minimum cluster size was set to three genes. The gene IDs from these clusters were extracted individually and tested for GO term enrichment using the functional annotations with TopGO (Rahnenfuhrer, 2022). This allows to characterize the molecular functions of these group of genes. To gain further insights into the expression differences of the ant DEGs, we investigated how the expression of the ant DEGs differed between the

three parasite load categories (uninfected, lowly infected and highly infected). To do so, we ran three LRT tests (same syntax of full and reduced models as described before) on a gene subset that only contained the ant DEGs: one for each of the three possible combinations of two of the three parasite load categories. Furthermore, we performed KEGG enrichment analysis by comparing the KEGG IDs of the transcriptome to the ant DEGs within different clusters using the clusterProfiler package on R (Wu et al. 2021). Finally, the transcriptome was BLASTed to a protein database for all invertebrates (downloaded on 10/08/2021) using the blastx algorithm of diamond BLAST (Buchfink et al. 2014) with an e-value cut-off of 1e-5. All BLAST hits per gene with the lowest e-value were extracted to identify which proteins the transcripts are potentially encoding. The individual gene functions of these BLAST hits were studied in the literature and using UniProt.

We processed the raw RNAseq data from our cestode samples in the same way as for the ants, except that we filtered out contaminating sequences from *T. nylanderi, H. sapiens, E. coli* and adapters, and vectors using FastQScreen. Furthermore, we used a transcriptome assembly of *A. brevis* (Stoldt et al. 2021) to map reads and based on this, estimated gene abundances. We also analysed the cestode gene count matrix using DESeq2, with a likelihood ratio test (LRT) to compare a model that included parasite load (lowly infected and highly infected) and colony identity as factors, to a reduced model including only colony identity. This produced a list of cestode genes with expression differences associated with the parasite load (cestode DEGs). We divided the cestode DEGs into upregulated and downregulated in the high parasite load category. The cestode DEGs in these groups were diamond BLASTed using the same invertebrate database as used for the ant DEGs and functionally annotated using TopGO. Gene functions were once again studied in the literature using UniProt. Furthermore, similar to the ant DEGs, we performed enrichment analysis on the extracted KEGG IDs from EGGNOG mapper using clusterProfiler. Finally, to investigate whether some of those genes encode for proteins that are secreted into haemolymph of the host ants (Hartke et al. 2023), we BLASTed (BLAST v.2.9.0; Altschul et al. 1990) the cestode DEGs against the RNA sequences of secreated cestode proteins.

We also compared gene expression between uninfected ants from infected colonies and uninfected colonies. When we plotted a PCA for the uninfected samples we found one sample in the uninfected colonies to be an outlier (Fig. S2). However, considering that we found no further indication that this sample was problematic (e.g., in our laboratory notes, sequence quality control), we included it in the analysis. Here we used the colony infection status as the variable factor using a Wald test on DESeq2 for a pairwise comparison of gene expression between different colony infection status (whether the sample came from an infected colony or not). Similarly, to the cestode DEGs, we split up the colony infection DEGs into overexpressed and underexpressed genes in the infected category. The colony infection DEGs were functionally annotated using TopGO and clusterProfiler and were BLASTed using diamond BLAST.

WGCNA analysis

We performed a weighted gene co-expression network analysis (WGCNA; Langfelder & Horvath, 2008) to investigate the effect of parasite load on gene networks. For this, we transformed the gene count

data using variance stabilizing transformation from DESeq2. Networks were constructed using all the ant samples including the samples from uninfected colonies. WGCNA was performed with a soft threshold power of 5 and a minimum module size of 250. To test whether parasite load affected gene expression of the constructed networks, we correlated the module expression to parasite load using Pearson's correlation and performed GO enrichment analysis on the significantly correlated modules.

Results

Influence of parasite load on morphology

Infected ant workers exhibited a smaller head width than uninfected ones (n=348, F= 33.442, p = 7.344e-09; Fig. 1a). Yet, worker head width did not further shift with increasing parasite load (n=268, F=0.536, p = 0.464; Fig. 1b). In contrast, the diameter of cestodes decreased with the logarithm of the number of cestodes in a single ant host (n=2850, F=391.31, p = 2.2e-16; Fig. 1 c).

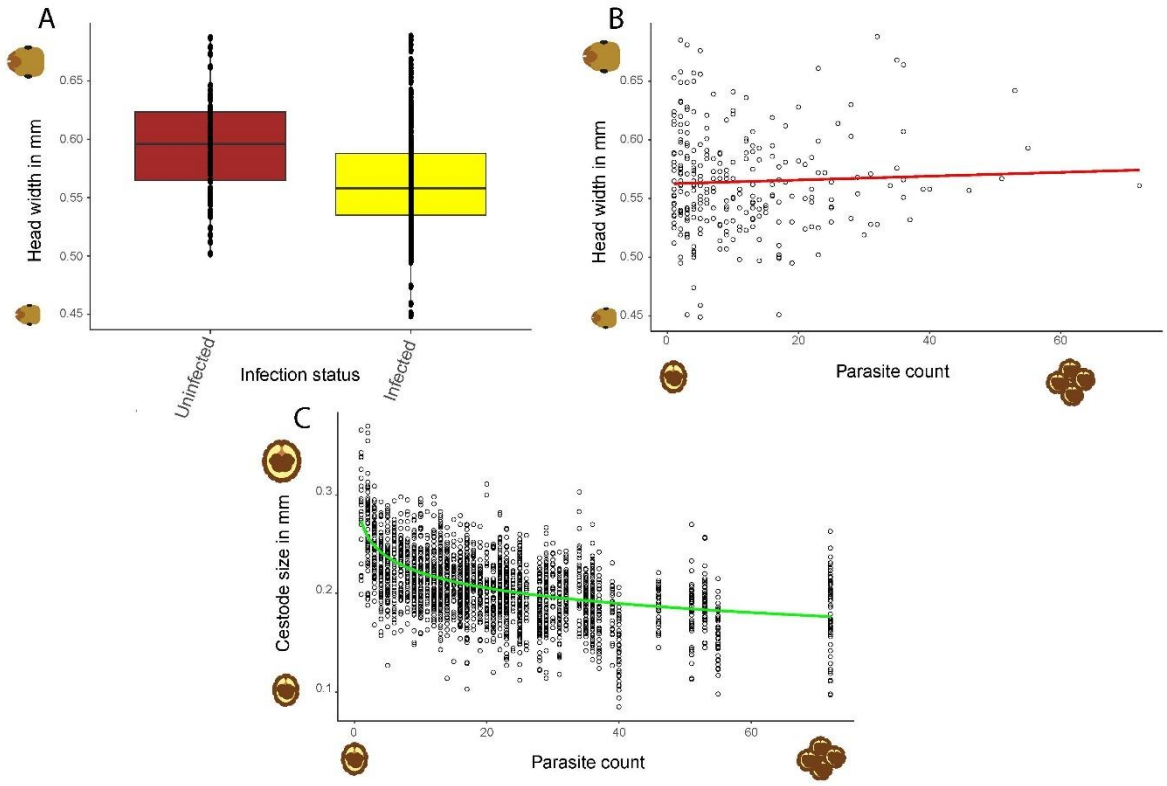

Figure 1 Changes in body size of parasites and hosts with parasite load. **A.** Infected workers had a smaller head width than uninfected workers (F= 33.442, p = 7.344e-09). **B.** No evidence for an association between head width of the host and parasite load (F=0.536, p = 0.464). **C.** Cestode size was negatively associated with parasite load (F=391.31, p = 2.2e-16).

Influence of colony infection status on gene expression of uninfected workers

Uninfected workers from infected colonies differed in the expression of 46 genes in the fat body from those from uninfected colonies. Of these genes, 28 were upregulated in workers from uninfected

colonies and 18 were upregulated in workers from infected colonies. Among the upregulated genes of workers from uninfected colonies, we found two significantly enriched GO terms represented by single genes, namely *transcription initiation by RNA polymerase* and *protein glycosylation*. The BLAST results revealed three homologues of probable cytochrome P450 6a14, which may have a function in insect hormone metabolism and synthetic pesticide degradation (Scanlan et al. 2020). For the overexpressed genes of workers from infected colonies, we found two enriched GO terms, also based on single genes *regulation of synaptic transmission, cholinergic* and *positive regulation of voltage-gated potassium channel activity*. However, the BLAST results provided limited information. Only three genes had annotations in *Drosophila melanogaster*, two of which were related to brain function and the last one was a transmembrane and TPR repeat-containing protein CG4050 that transfers mannosyl residues to the hydroxyl group of serine or threonine residues (Larsen et al. 2017).

Influence of parasite load on ant gene expression

We identified 122 differentially expressed genes (ant DEGs) in the fat body, whose expression varied according to infection status and/or parasite load. The cluster analysis grouped 120 of these ant DEGs into three clusters with different expression patterns. Cluster 1 consisted of 62 genes with high expression in highly infected workers, while the uninfected and lowly infected workers showed low expression of these genes (Fig. 2a). These genes were enriched for *oxidative stress response* (Fig. 2d). Pairwise comparisons revealed that 17 of these genes were differentially expressed between lowly and highly infected ants. In addition, ten more genes differed in expression between highly and uninfected workers than between lowly and uninfected workers (Table S3). Genes of note in this cluster include two homologues of genes encoding the dual-oxidase II (Fig. 3a and b). Both differed significantly between highly infected and uninfected workers, with low-infected workers not differing significantly from either. This protein mainly has been shown to play a role in the innate immunity, limiting microbial proliferation in *Drosophila melanogaster* guts (Ha et al. 2005; Lee et al. 2015). Other genes of note encode tyrosine-protein kinase Src64B, which may be involved in the development of neural tissue and smooth muscle (Simon et al. 1985) as well as salivary gland development (Sopko & Perrimon, 2013). Furthermore, there were three proteins involved in eye development, including protein tincar (Fig 3c; Boulton et al. 2000; Hirota et al. 2005; Rahman et al. 2012), which differed in expression between highly infected and uninfected workers, while there were no expression differences to the low-infected group in both cases.

The second cluster included 47 DEGs (Fig. 2b) and as the graphical representation indicates that these genes were generally upregulated in uninfected workers and downregulated in both groups of infected workers. Nevertheless, pairwise comparisons revealed fourteen genes that differed in expression between lowly and highly infected workers and nine additional DEGs between highly and uninfected workers that did not differ between uninfected and lowly infected workers (Table S3). Among the enriched GO terms of this cluster, we found *activation of innate immune response*. From the BLAST results, genes of interest include those encoding the cytochrome c oxidase subunit 5A (Fig. 3d), which is the last enzyme in the mitochondrial electron transport chain, driving oxidative phosphorylation (Hartley et al. 2019) and therefore plays an important role in energy metabolism.

Finally, cluster 3 contained 11 DEGs, which were downregulated in the uninfected workers but upregulated in the lowly and highly infected workers (Fig. 2c). For none of these genes differed in expression between the lowly and highly infected group (table S3). Only one GO term was found to be enriched, which was *protein glycosylation*, based on a single gene. Genes of interest include one encoding a dopamine N-acetyltransferase (Fig. 3e), which regulates sleep homeostasis by limiting the accumulation of serotonin (Brodbeck et al. 1998; Davla et al. 2020; Shaw et al. 2000).

We found no significantly enriched KEGG IDs for any of the clusters. In the WGCNA analyses, we identified a total of 10 modules with a minimum module size of 100. However, none of these clusters were significantly associated with parasite load (Fig. S3).

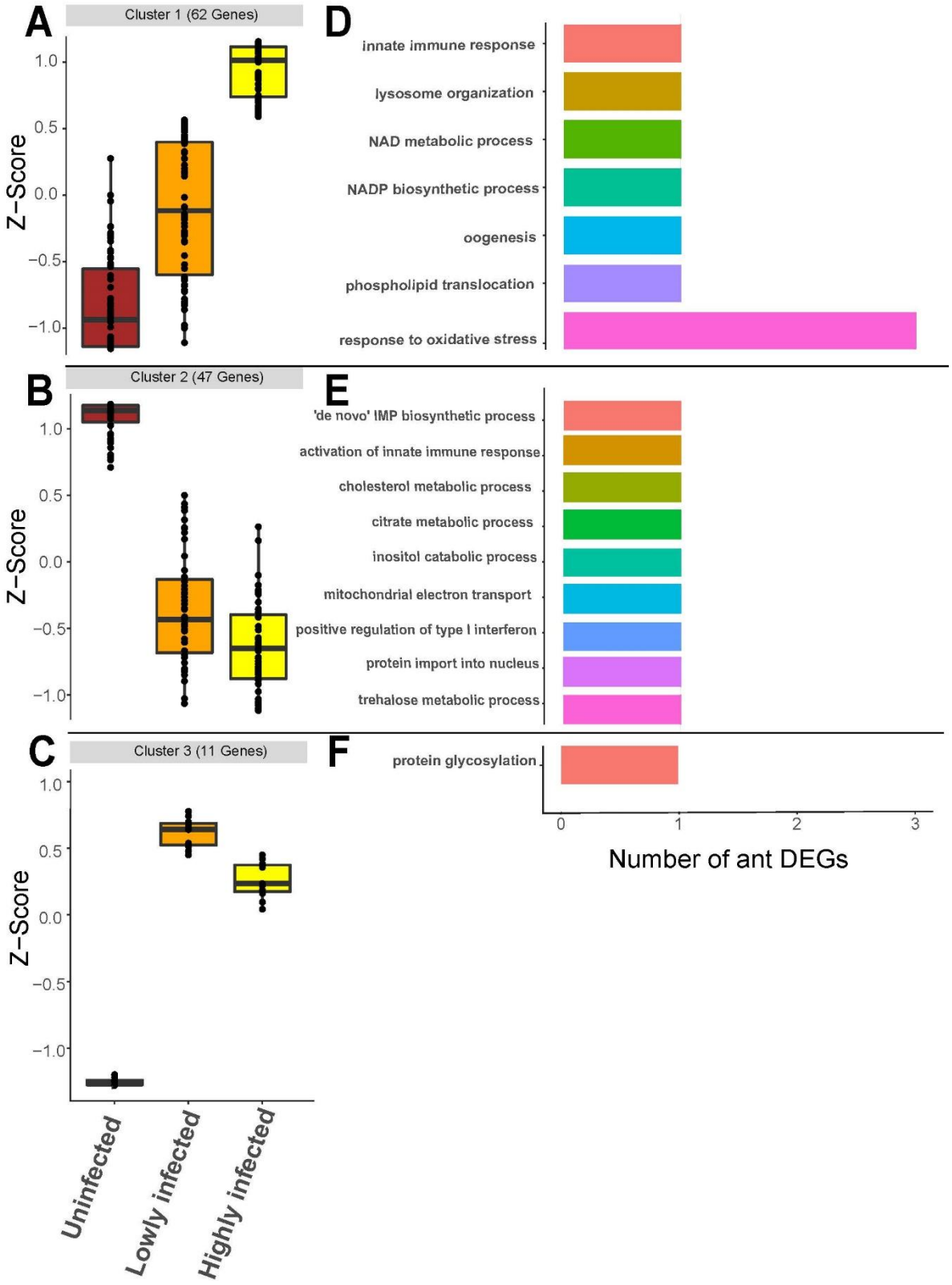

Figure 2 Clustering and visualization of 120 (out of 122) of the ant DEGs. The genes could be grouped into three clusters according to their expression patterns: **A.** Cluster 1, **B.** Cluster 2 and **C.** Cluster 3. GO enrichment analyses of these clusters (**D** for cluster 1, **E** for cluster 2 and **F** for cluster 3) were calculated with topGO using the weight01 algorithm and the GO annotations of the biological processes of the clusters were compared with the whole transcriptome using Fisher's exact test. Each bar represents a significantly enriched GO term in each cluster, the x-axis represents the number of significant genes.

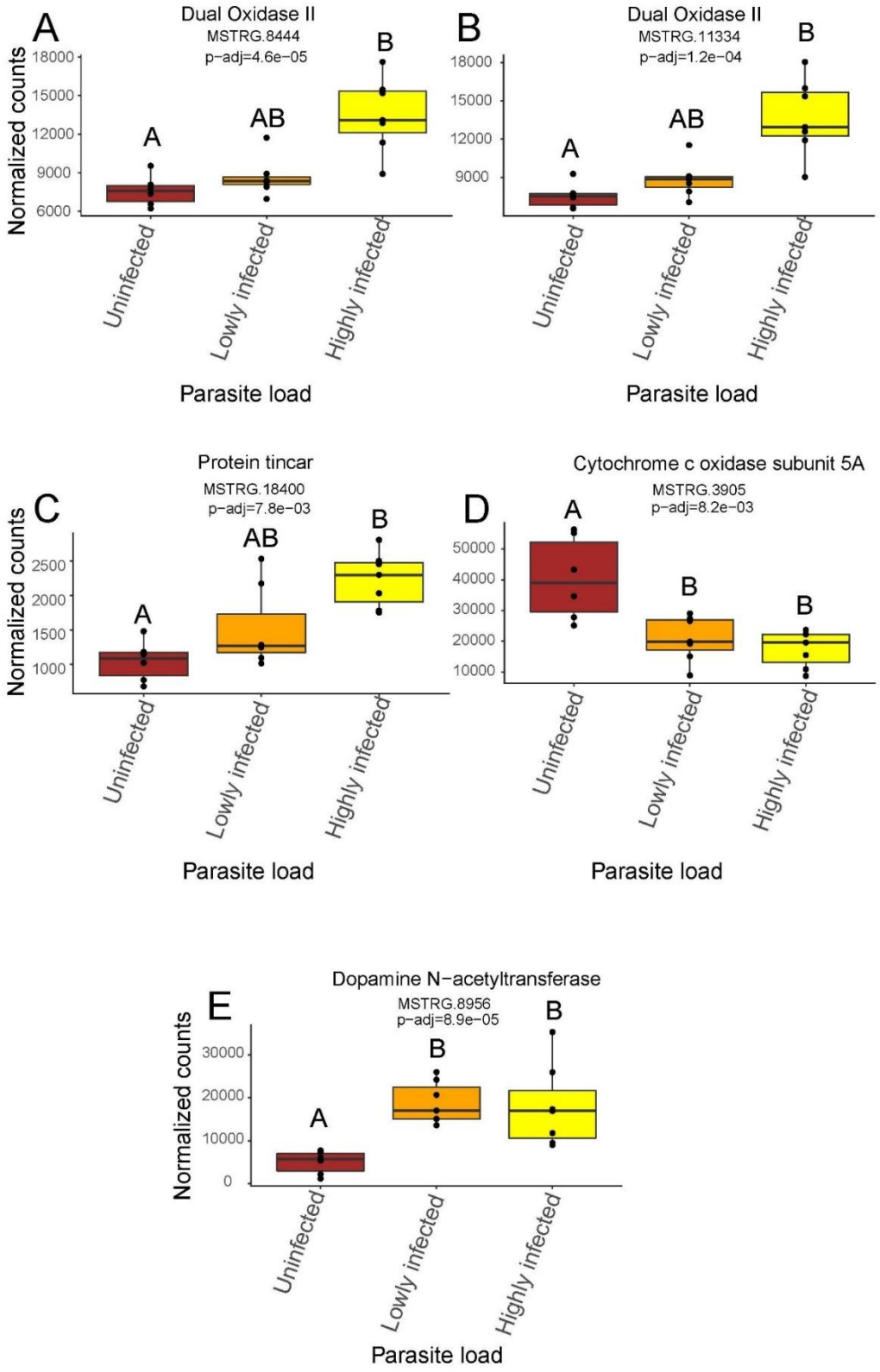

Figure 3 Selected ant DEGs with notable functions. **A-C** shows DEGs from cluster 1, which were upregulated in highly infected individuals (but not in lowly infected ones) compared to the uninfected individuals. **D** belonging to cluster 2, upregulated in uninfected workers. **E** belonging to cluster 3, with DEGs downregulated in uninfected workers.

Influence of parasite load on cestode gene expression

Analysis of the cestode dataset revealed 377 DEGs between cestodes from highly and lowly infected ants (cestode DEGs), of which 357 were upregulated in cestodes co-infecting a single host with many others.

Ten highly expressed genes in this group are involved in the serine/glycine interconversion pathway. Serine proteinases are associated with multiple functions in cestodes, including defence against the host immune system (Izvekova et al. 2021; Patel, 2017) and lipid metabolism (Webb & Mettrick, 1975). In addition, we found overexpression of mitochondrial uncoupling protein 4, which is involved in the response to starvation in *C. elegans* (Fig. 4a) and regulates the long-chain fatty acid metabolism, primarily in response to nutrient availability. The production of these lipid-signalling proteins regulates several processes that may control lifespan and adaptation to starvation (O'Rourke et al. 2013). Finally, we found an upregulation of a gene encoding a vesicular glutamate transporter, which in *C. elegans* is required for the detection of preferred food sources (Harris et al. 2014). The GO enrichment analysis (Fig. 4c) of these upregulated genes in parasites from highly infected hosts revealed transport functions, among which are *carbohydrate transmembrane transport, regulation of pH* and *sodium ion transport.* When comparing these genes to those proteins released into the haemolymph of host ants (Hartke et al. 2023) using BLAST, we found three matching genes, none of which were annotated.

Among the 20 genes upregulated in parasites residing alone or with one other parasite in a host, we found a gene encoding a Lon protease (Fig. 4d), which is a serine-dependent protease that ensures the maintenance of the integrity of the mitochondrial genome (Kaunisto & Suhonen, 2013; Nargund et al. 2012). In the GO enrichment of upregulated genes in cestodes from lowly infected hosts, we found *vesicle-mediated transport* as a significantly enriched function, as well as *carbohydrate transmembrane transport* (both 2 genes) and fructose 6-phosphate metabolic process (Fig. 4e), both involved in the absorption of nutrients. No significantly enriched KEGG IDs were found in the entire dataset. Finally, we detected one BLAST match with a secreted cestode protein in the DEGs, but again the gene had no annotation.

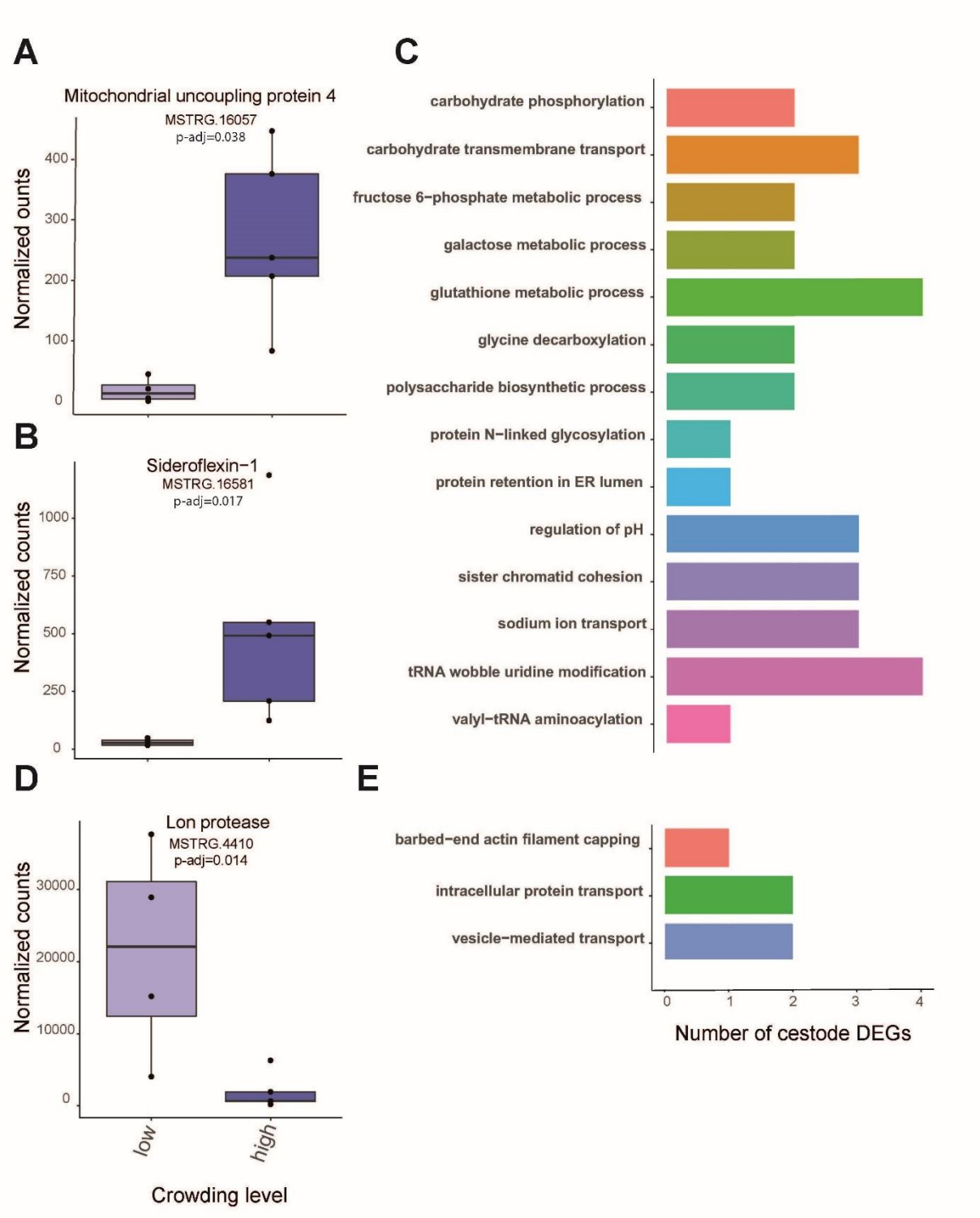

Figure 4 Changes in gene expression in cestodes from highly infected workers. **A**, **B** and **D**: Expression differences for three selected cestode DEGs. **C.** Barplots representing the GO terms for the genes that were upregulated with higher parasite load. **E.** Barplots representing the GO terms for the genes that were upregulated with lower parasite load.

Discussion

The aim of our study was to investigate whether parasite load by the cestode *A. brevis* affects gene expression and morphology of *T. nylanderi* ant workers. About a quarter of all 120 clustered differentially expressed genes in the host fat body responded to parasite load, and in heavily infected workers, genes responsive to oxidative stress function were particularly upregulated. The cestodes were also strongly influenced by crowding. Our transcriptomic results indicate that co-infecting parasites suffer from poorer access to nutrients as reflected by their smaller body size.

Influence of parasite load on host gene expression

Both pairwise comparisons as well as clustering of differentially expressed genes (DEGs) in ants revealed that the expression of many genes responded to parasite load, whereas others responded to infection in an all-or-nothing manner such as those DEGs grouped in cluster 3. Looking at the GO enrichment of cluster 1, the most enriched term was *oxidative stress response*. A higher parasite load could be responsible for more reactive oxygen species, i.e., more oxidative stress, as has been shown in rodents infected with a high load of the protozoan parasite *Trypanosoma cruzi* (Vilar-Pereira et al. 2021) or in erythrocytes infected with malaria (Becker et al. 2004). However, increased response to oxidative stress could also be a way to extend lifespan (Ristow & Schmeisser, 2011). Infected *T. nylanderi* host workers live far longer than their uninfected nestmates (Beros et al. 2021), and this may be explained in part by a stronger response to oxidative stress. In our system, the parasite might benefit from the host's life extension, as this increases the chances that the final host, a woodpecker, will prey upon the infected ant. None of the genes in clusters 2 and 3 were involved in the regulation of oxidative stress. Based on the expression plots (Fig. 2) and the pairwise comparisons, we interpret that the enrichment detected in cluster 1 is mainly due to the large number of DEGs that differ between uninfected and lowly infected workers on the one hand and highly infected workers on the other, whereas hardly any differences were found between uninfected and lowly infected workers. This would mean either that only a high parasite load may trigger oxidative stress in the host, to which the ant responds by upregulating the response genes, or that only many parasites jointly can influence the host to upregulate oxidative stress genes and thus live longer. To date, it has not been studied how parasite load relates to the longevity of individual workers, and we plan to investigate this. We would argue that it is more likely that the oxidative stress response is a side-effect of parasite load or a consequence of heightened immune response of the host, as higher parasite burden has been linked to a high oxidative stress response in other parasite-host systems (De Coster et al. 2012; Stocker et al. 1985). The higher stress from infection is also supported by the differential innate immune response, which we find confirmed in the GO term analysis. Another interpretation for the upregulation of oxidative stress response genes could be the fact that heavily infected ants are older than workers with a lower parasite load. Since we have collected these ants as already infected adults in the field, we have no information about their age. However, there is no evidence that cysticercoids of *Anomotaenia* can divide in their intermediate host (Gabrion & Helluy, 1982), which would suggest a higher age of heavily infected ants. In other systems, ageing itself has been shown to lead to higher levels of oxidative stress (Liguori et al. 2018; Lin & Beal, 2003). For example, older *Temnothorax* queens overexpress genes with response to oxidative stress functionality (Negroni et al. 2019), but the relationship between senescence and oxidative stress response in social insects is far from consistent (Kramer et al. 2021). Although infection leads to lower activity in workers,

it is currently unclear whether activity continues to decrease with parasite load. In fire ants, the expression of response to oxidative stress genes is linked to foraging activity (Manfredini et al. 2014), so there may be an association here, yet one would expect an inverse link: lower activity in heavily infected animals leads to fewer reactive oxygen species and a lower response. Finally, it is possible that oxidative stress response gene activity varies with parasite load, as highly infected ants have a higher nutrient intake. Infected workers are fed more often compared to uninfected ones (Beros et al. 2015), but a relationship with parasite load has not yet been investigated. However, higher nutrient intake been shown to increase reactive oxygen species production and to accelerate aging (Metcalfe & Alonso-Alvarez, 2010; Sohal & Weindruch, 1996). To address this issue, we propose to further investigate the social care of highly infected workers compared to lowly infected workers.

While examining the functions of differentially expressed genes, a gene of interest was one encoding *dual oxidase II*, two homologues of which were upregulated in highly infected workers (but not in lowly infected ones) compared to uninfected workers. *Dual oxidase II* is involved in limiting microbial proliferation in the gut of *D. melanogaster* flies (Ha et al. 2005; Lee et al. 2015), suggesting possible changes in the gut microbiome of highly infected workers. In general, the gut microbiome of *T. nylanderi* appears to be colony-dependent, with little difference between castes, although colonies with a greater microbial diversity are more productive (Segers et al. 2019). A difference in the gut microbiome caused by cestode infection might be linked to parasite load, and this should be investigated further.

As we did not perform experimental infections, it is possible that differences in parasite load are due to existing genetic differences in ant hosts or parasites. Perhaps some host genotypes are able to fight *Anomotaenia* larvae more successfully at an early stage of infection, or some cestode genotypes are better able to overcome host defences. This is also supported by the fact that the proportion of infected animals per colony varies considerably. In one colony from our study area, 70% of all workers were infected, in other colonies only single workers were infected. In addition to differences in exposure to parasite eggs, this is could be due to genetic differences between the parasites or the ant colonies. However, we controlled for those by analysing one uninfected, one lowly and one highly infected worker per colony. In addition, *T. nylanderi* ants have a restricted foraging radius of about half a metre (Heinze et al. 1996) and it is unlikely that foragers encounter a lot of bird droppings that could contain cestode eggs. We therefore deem it reasonable to assume that the cestodes within single colonies have a high degree of relatedness as well since they probably came from the same patch of bird excrement. Finally, we avoided using colonies with an infection rate that is too low or too high and we restricted our infected worker representation between 6% and 22%. However, all this does not completely rule out genetic effects, as there could be genetic differences in the susceptibility to or infectivity of *A. brevis* within a colony.

Confirming previous results (Feldmeyer et al. 2016; Stoldt et al. 2021), we identified an enrichment for innate immune response in cluster 2, containing genes with high expression in uninfected workers. However, we should note that this GO enrichment was based on a single gene. Another notable difference between infected and uninfected ants is the number of differentially expressed genes involved in transport processes. Examples of enriched GO terms include *mitochondrial electron transport* and *protein import into the nucleus*, both represented by a single gene. In addition, we found

genes encoding proteins with transport functions, such as cytochrome c oxidase subunit 5A (Hartley et al. 2019) and cystinosin homologues isoform X3 (Yu et al. 2008). Differential expression of genes involved in transport is frequently observed during parasite infection (Berger et al. 2021; Kremer et al. 2013). Transport genes are thought to be involved in cestode-host communication, and more commonly as a means of manipulating host phenotypes (Berger et al. 2021). Thus, the upregulation of transport genes may indicate active manipulation by *A. brevis*, which should be investigated further, even though we found no evidence in cluster 1 that heavily infected ants particularly upregulate transport genes. A final candidate gene in cluster 3 encodes a dopamine N-acetyltransferase gene involved in sleep regulation in insects (Brodbeck et al. 1998; Davla et al. 2020; Shaw et al. 2000). Its upregulation in lightly and heavily infected workers could possibly explain why infected ants are so inactive. Biogenic amines such as dopamine are important in the brain for regulating behaviour. So, if the parasite is interfering with host gene expression in the fat body, it is probably doing so in the wrong tissue. However, it has already been shown in other parasite systems that parasites do not always exclusively influence the physiology of tissues they are located in (Laothamatas et al. 2003). Inhibition of this gene using RNAi may help to understand whether it indeed plays a role in the behavioural changes of infected animals (Berger et al. 2021; Kremer et al. 2013).

Parasite load was unrelated to host body size, which is in contrast to previous results demonstrating a negative correlation between ant head size and parasite load (Scharf et al. 2012). The discrepancy may be due to different methodologies: Scharf et al. (2012) measured the entire head surface, while we used only head width a common measure of body size in ants. It is possible that increasing parasite load causes a shortening of the head but does not cause a reduction in head width from eye to eye. This might be related to a reported atrophy of the mandible closer muscle in infected ants (Feldmeyer et al. 2016). Changes in head shape have not been studied in relation to parasite load, but is a known consequence of parasite infection in various systems (Johannessen, 1973; P. T. J. Johnson & Sutherland, 2003). Our measurements did demonstrate a clear reduction in head width related to the cestode infection *per se.* This would mean that head width falls into our second category of phenotypic changes, those that shift with infection in an on/off manner.

Influence of crowding on cestode morphology and gene expression

We can show that crowding (multiple cestodes in a single host) leads to reduced body size in cysticercoid larvae of our parasitic cestode. This has been previously noted in *A. brevis* (Scharf et al. 2012) and several other cestode species (Benesh, 2019; Heins et al. 2002) and may indicate reduced nutrient availability for cestodes residing in heavily infected workers. This was also evident in the transcriptional shifts observed with crowding. When looking at the functional annotation of the cestode DEGs, many functions related to transport become apparent, similar to the ant dataset. For parasites living under crowded conditions, these include *carbohydrate transmembrane transport* (3 genes), *pH regulation* (3 genes) and *sodium ion transport* (3 genes). These enriched GO terms could indicate the need to transport in nutrients and salts and to regulate the pH. In contrast, parasites infecting an ant host alone upregulated DEGs enriched for *intracellular protein transport* (2 genes) and *vesicle-mediated transport* (2 genes). Cestodes are known to release vesicles into their environment that contain miRNAs (Ancarola

et al. 2020, 2017). In our study system, tetraspanin, a protein considered indicative of extracellular vesicles, was identified among the cestode proteins released into ant haemolymph, suggesting that *A. brevis* uses this pathway to interact with its host (Hartke et al. 2023). A function of extracellular vesicles in parasite-host communication or manipulation has been suggested also in other systems (Coakley, et al. 2015; 2016; Siles-Lucas et al. 2015; Tritten & Geary, 2018), this all points to a need of our parasites living alone to communicate more with (or manipulate) their hosts. A GO term supported by four genes for cestodes living under crowded condition was glutathione metabolic process. An upregulation in glutathione provides protection from and tolerance of severe stress (Maher, 2005), again indicating physiological stress. Ten serine proteases that are upregulated in cestodes living under crowded conditions provide more insights. Among other things, serine proteases are related to the ability of cestodes to evade the host immune system (Izvekova et al. 2021; Patel, 2017). Since infection with multiple parasites could trigger a stronger host immune response, this enhanced evasion of the host immune system might be necessary. Under crowded conditions, there may also be competition for food among parasites. Indeed, we find evidence for this. For example, mitochondrial uncoupling protein is upregulated in co-infecting cestodes, which has been shown to allow at least *C. elegans* to adapt to food deprivation (Folick et al. 2015; Lapierre et al. 2011; O'Rourke et al. 2013; Ramachandran et al. 2019). Although these effects could generally be considered negative for the parasite, nutrient stress fits surprisingly well with the oxidative stress experienced by the host during high parasite loads, as oxidative stress is inherently life-prolonging (Ristow & Schmeisser, 2011). This is mainly because low food intake has been shown to prolong life in both fungi (Lin et al. 2000) and animals (Mathis et al. 2017). In this context, it should not be forgotten that the parasite must live for a very long time as well; it is not enough to increase the life expectancy of the host to several years; the cysticercoid larvae also have to stay alive until they reach their final host. If they do so and have lived with other cestodes in the intermediate host, they are also likely to co-infect their final avian host, which could lead to a reduction in fitness during their reproductive phase, although a fitness reduction has not been shown yet because of crowding (Benesh, 2019). The consequences of crowding are thus often manifold and lasting.

Conclusion

Not only the infection *per se* but also the parasite load can cause multiple consequences in the physiology for both the host and the parasite. In the host of our study system, we found that many of the differentially expressed genes respond to parasite load. Gene functions were indicative of oxidative stress and innate immunity, suggesting general stress and possibly also extended lifespans in highly infected hosts. We also find evidence that sole parasites invest more in vesicular transport mechanisms. On the other hand, crowded parasites seem to suffer from nutrient stress and invest more in the evasion of the hosts' immune system, nutrient transport, and stress resistance. These deeper insights into the host-parasite interaction have demonstrated that both the parasite and the host are affected by changes in parasite load.

Acknowledgements

We would like to thank [redacted] for creating the illustrations of *T. nylanderi* and *A. brevis*, as well as designing Figure S1. Furthermore, thanks go to [redacted] for her help with the dissection setup and [redacted] for doing some of the RNA isolations needed for this study as well as her instructions on how to do them. Finally, we would like to thank [redacted] for their input and fruitful discussions regarding the data analysis. This project was funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) – GRK2526/1 – Projectnr. 407023052.

Data availability

Raw reads used for this study are available on NCBI under the submission ID SUB12766169. Scripts on processing raw reads, R scripts for analysis, gene count matrices, pictures used for morphological analysis, BLAST hits and InterProScan output can be found on Mendeley on the DOI 10.17632/x3kxjddv9d.1.

Supplementary materials

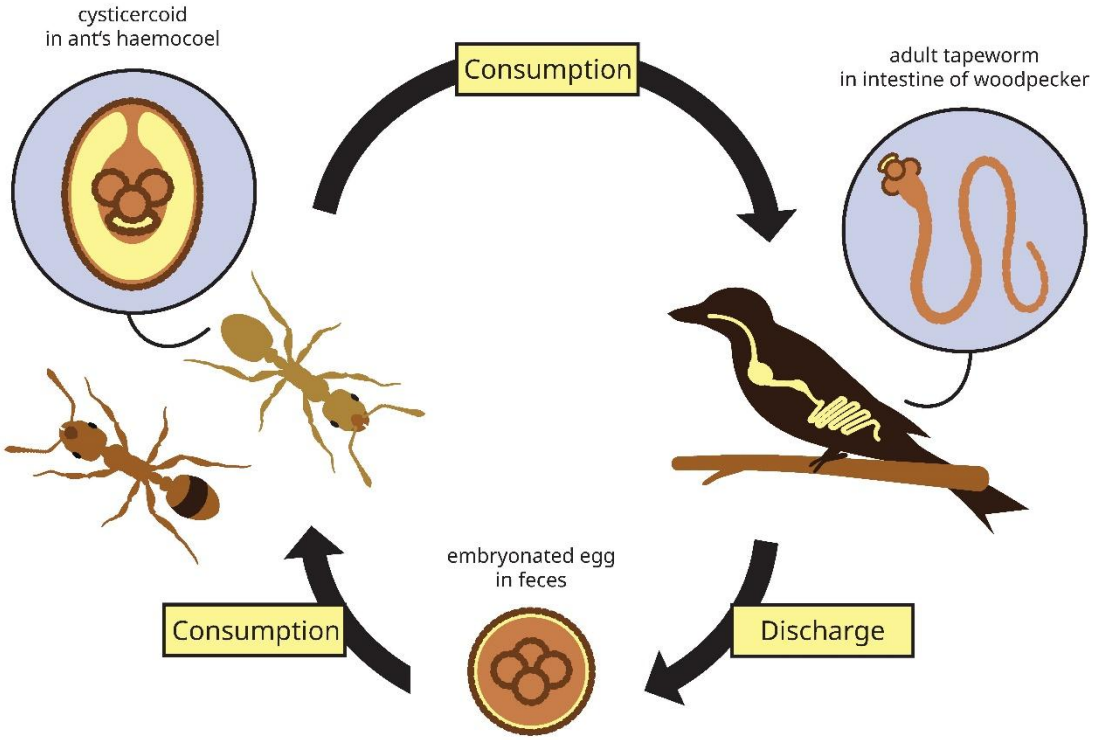

Figure S1 A visualisation of the life cycle of *Anomotaenia brevis*. Their eggs are located in the discharge of woodpeckers which is consumed by *Temnothorax nylanderi*, where *A. brevis* spends its cysticercoid stage. When the ants are then consumed by woodpeckers they grow into their adult life stage.

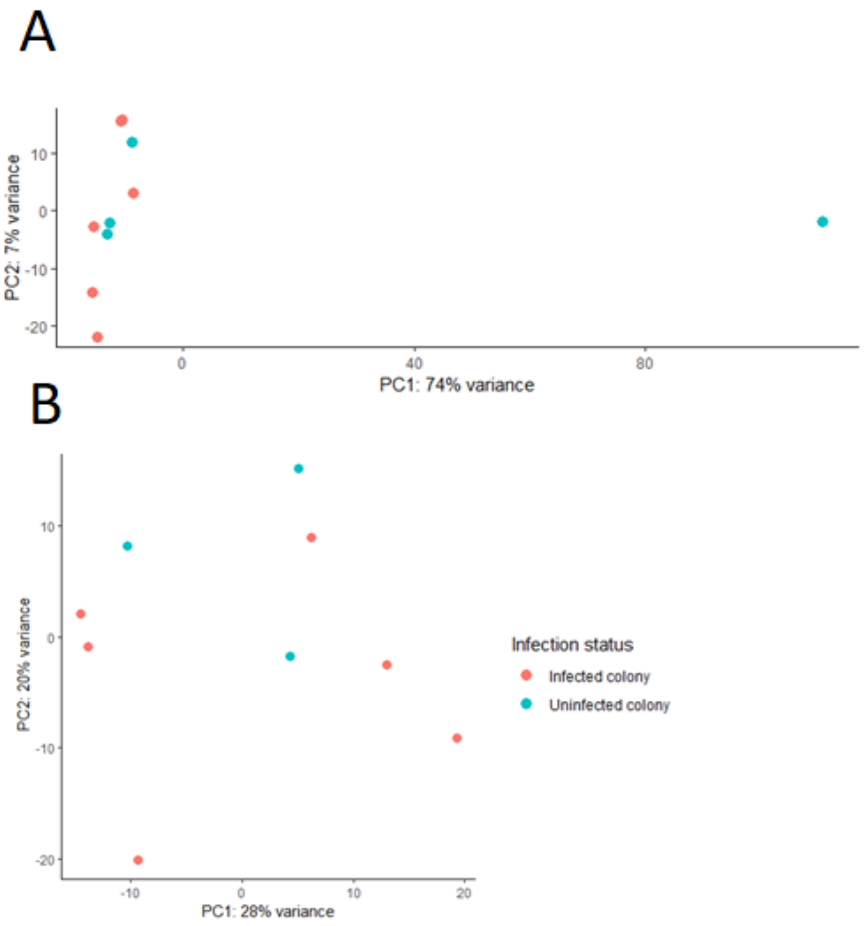

Figure S2 A. PCA of the uninfected colonies where the colour represents infected (red) or uninfected (blue) colonies. No clustering based on infection status was observed in the data, however, one uninfected sample played a large role in PC1, which explains 74% of the variance. **B.** PCA of the uninfected colonies with the outlier removed. Once again no clustering is observed in the data based on infection status.

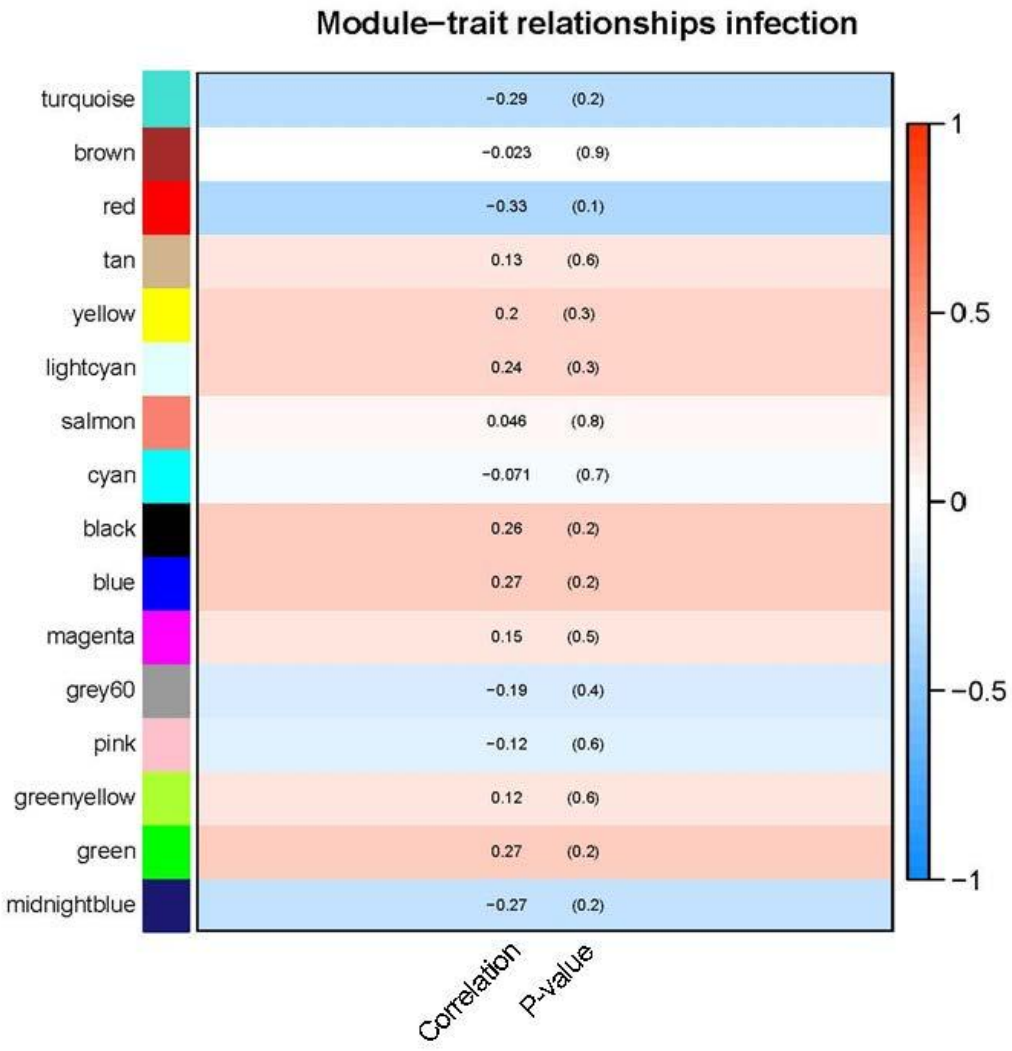

Figure S3 Heatmap of the different WGCNA modules, which are indicated with the colour names with their correlation coefficient (signified by “Correlation”) and p-value (signified by “P-value”). None of the modules correlate significantly with parasite load.

Table S1: Overview of the colonies used for the analysis. The colonies marked in bold were used for gene expression analysis of the ants. From colonies indicated by an asterisk**,** we extracted the RNA from cestodes as well

Colony identity	**Year of collection**	**Number of queens**	**Total number of workers**	**Number of infected workers**	**Infection rate**	**Uninfected workers dissected**
LBW20TS15	2020	1	77	31	0.40	7
LBW20TS49*	2020	1	65	4	0.06	3
LBW20TS52*	2020	1	44	16	0.36	4
LBW20TS58*	2019	1	164	16	0.10	5
LBW20TS61	2019	1	89	1	0.01	2
LBW20TS86	2019	1	65	2	0.03	1
LBW20TS89	2019	1	93	5	0.05	2
LBW20TS90	2019	1	129	3	0.02	2
LBW20TS97	2020	1	196	3	0.01	2
LBW20TS106*	2020	1	72	16	0.22	4
LBW20TS107	2020	1	137	4	0.03	2
LBW20TS108	2020	1	177	126	0.71	26
LBW20TS113*	2020	1	121	24	0.20	4
Lbw20TS117	2020	1	62	1	0.02	2
LBW20TS118	2020	1	86	1	0.01	2
LBW20TS120	2020	1	16	2	0.13	2
LBW20TS121	2020	1	72	8	0.11	2
LBW20TS132	2020	1	88	1	0.01	2
LBW20TS145	2020	1	17	2	0.12	2
LBW20TS149	2020	1	76	8	0.11	2
LBW20TS153	2020	1	66	1	0.02	2
LBW20TS9	2020	1	68	0	0	5
LBW20TS21	2020	1	167	0	0	5
LBW20TS99	2019	1	99	0	0	5
LBW20TS105	2020	1	49	0	0	5

Table S2 Overview of all samples used for RNA seq analysis and time and day of sampling. Samples marked with an asterisk are from ants and cestodes that have representatives in the other datasets. For example, cestode sample B5 comes from the same ant as the fat body in ant sample B5. The sample marked in grey (C16) was removed from analysis due to poor read quality.

Highly infected workers

Code	Colony	Infected	Date	Time	Parasite count
J2*	LBW20TS106	y	30-7-2020	18:40	23
M19*	LBW20TS113	y	4-8-2020	10:30	18
S1	LBW20TS145	y	31-7-2020	15:10	34
T3	LBW20TS149	y	6-8-2020	13:15	28
D16*	LBW20TS58	y	31-7-2020	14:00	51
G5	LBW20TS89	y	4-8-2020	10:05	31
B5*	LBW20TS49	y	28-7-2020	15:00	29

Lowly infected workers

Code	Colony	Infected	Date	Time	Parasite count
J10	LBW20TS106	y	4-8-2020	13:45	1
M7	LBW20TS113	y	29-7-2020	13:25	1
S2	LBW20TS145	y	7-8-2020	09:20	3
T10	LBW20TS149	y	29-7-2020	18:30	5
D15	LBW20TS58	y	4-8-2020	09:50	2
G7	LBW20TS89	y	5-8-2020	13:15	6
B3	LBW20TS49	y	24-7-2020	14:00	2

Uninfected workers from infected colonies

Code	Colony	Infected	Date	Time	Parasite count
J19	LBW20TS106	n	3-8-2020	19:00	0
M24	LBW20TS113	n	31-7-2020	16:35	0
S5	LBW20TS145	n	6-8-2020	10:40	0
T11	LBW20TS149	n	6-8-2020	15:50	0
D20	LBW20TS58	n	7-8-2020	11:40	0
GX	LBW20TS89	n	10-8-2020	17:50	0
B6	LBW20TS49	n	7-8-2020	15:00	0

Uninfected colonies

Code	Colony	Infected	Date	Time	Parasite count
V1	LBW20TS9	n	5-8-2020	13:05	0
W3	LBW20TS21	n	4-8-2020	15:25	0
X4	LBW20TS99	n	10-8-2020	11:35	0
Y2	LBW20TS105	n	6-8-2020	17:35	0

Cestodes

Code	Colony	Infected	Date	Time	Parasite count
B4	LBW20TS49	y	21-7-2020	17:55	1
B5*	LBW20TS49	y	28-7-2020	15:00	29
C16	LBW20TS52	y	4-8-2020	19:15	1
C9	LBW20TS52	y	31-7-2020	09:30	22
D15	LBW20TS58	y	10-08-2020	09:25	2
D16*	LBW20TS58	y	31-7-2020	14:00	51
J10	LBW20TS106	y	4-8-2020	13:45	1
J2*	LBW20TS106	y	30-7-2020	18:40	23
M11	LBW20TS113	y	5-8-2020	09:20	1
M19*	LBW20TS113	y	4-8-2020	10:30	18

Table S3 Overview of the pairwise comparisons between ant DEGs

Cluster ID	No. of genes in cluster	Uninfected v lowly infected	Uninfected vs highly infected	Lowly infected vs highly infected
Cluster 1	62	33 (53%)	43 (69%)	17 (27%)
Cluster 2	47	38 (81%)	47 (100%)	14 (30%)
Cluster 3	11	11 (100%)	9 (82%)	0 (0%)

Chapter 2

Tapeworm infection and a sham bacterial challenge result in independent changes in transcriptional activity and gut microbiome composition in a social insect

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In preparation

Author contributions

T.S. and [redacted] conceptualized and designed the original study with inputs from [redacted], sample collection was done by **T.S.** and [redacted]. Experiments were performed by **T.S.**. Transcriptome analyses were done by **T.S.**, while gut microbiome analysis was a combined effort of **T.S.** and [redacted]. The original draft was written by **T.S.**. The manuscript was edited by **T.S.** and [redacted] with help of [redacted].

Abstract

Animal immune systems respond to different types of pathogenic infections. Although immune response pathways can be highly specific, they are often interlinked. Not only the immune system of multicellular organisms react to immune challenges, but also their symbionts can be involved in host immunity, e.g., the gut microbiome. Here we investigate how a social insect, the ant Temnothorax nylanderi, responds to infection with the tapeworm Anomotaenia brevis and simulated bacterial immune challenge using lipopolysaccharides (LPS) of gram-negative bacteria. Our experimental design allows us to examine not only main effects, but also their interaction on two interrelated metrics: transcriptional activity in the fat body, an organ important for immune defence, and the composition of the gut microbiome. We demonstrate strong responses to both challenges, with more profound consequences of the persistent cestode infection, but no evidence of an interaction, either in the gut microbiome nor in the transcriptome analysis. However, we uncover that cestode infection, and a sham bacterial challenge shift the expression of commonly targeted immune genes in opposite directions. The cestode appears to be able to suppress the host's immune response against itself without weakening it against other pathogens. We have identified several immune pathways involved in basal and induced immune responses in this social insect including glutathione metabolism and the Toll pathway. Taken together, our study shows that the same immune genes can respond to the presence of different types of pathogens, but these effects might not be synergistic and can go in opposite directions.

Keywords: host-parasite interaction, gut microbiome, manipulation, immune reaction, cestode

Introduction

The evolutionary arms race between hosts and internal parasites or pathogens often focuses on the strength of the hosts' immune response (Anderson et al. 2010; Spottiswoode & Busch, 2019; Zhao & Guo, 2019). From a parasite's perspective this means that it either needs to be able to evade host immune system recognition (Ezema et al. 2023; Schmid-Hempel, 2008, 2009) or suppress the immune response of the host (Chulanetra & Chaicumpa, 2021). However, complete inhibition of the host's immune system is usually not beneficial for parasites that cause persistent infections, since they need to keep their hosts alive for as long as they are dependent on them. This period can range from the entire lifespan of the parasite to the moment when the parasite can transmit from an intermediate host to the definite host, or until a parasite or parasitoid can develop into a free-living stage. When parasites cause persistent infections, as is often the case with helminths (Beros et al. 2021; Le et al. 2020; Van Riet et al. 2007) or protozoa such as *Toxoplasma* (Bisoffi et al. 2016) and *Trypanosoma* (Meurs et al. 1998), they should ensure that the host lives long enough for the parasite to reproduce or produce infective stages, most obviously seen in jewel wasps as they paralyze their host while their larvae target non-essential organs to consume first (Catania, 2020). This also counts for conventional parasites that do not kill their host as other parasites may attack the host during this time and these infections may have devastating consequences, an effective immune system should be maintained against these competing or harmful parasites.

These persistent parasites often use species-specific strategies to evade the host's immune response. In some species we find partial immunosuppression, where the parasite is able to attack specific cell types or proteins involved in the immune response directed against it (Heil, 2016; Noël et al. 2004; Regan et al. 2014). This is difficult because there are also generalised immune reactions, such as inflammation and the recognition of foreign intruders (reviewed in Chaplin, 2010). In some cases, however, the parasites appear to target the general immune response. This is the case with *Echinococcus* species, which target the host T-helper cytokines involved in the inflammatory response, while activation of Th cytokines in early stages of cestode infection has been shown to kill the parasite (Reviewed by Vuitton, 2003). Such immune suppression by helminths can make a host prone to be infected by other pathogens, such as by *Plasmodium* resulting in malaria (Hartmann et al. 2011; Mabbott, 2018; Scott, 2017). This would mean that a parasite would have to share a host with others leading to competition for host resources (Mideo, 2009) or in the case of very virulent parasites/parasitoids, premature death of the host and thus severe fitness costs of the original parasite. Viruses and bacteria usually use the alternative strategy of evading the immune system (Schmid-Hempel, 2008). *Plasmodium*, the parasitic protozoan causing malaria secretes a number of proteins that bind to immunoglobulin M, which makes it harder for immune cells to bind to malarial antigens, thereby evading the immune response (Ji et al. 2023). This molecular mimicry comes with a trade-off for the parasite in that it potentially induces an autoimmune reaction in the host and compromises protein functioning, lowering the within-host replication rate and leading to fewer new infections (Hurford & Day, 2013).

Parasite-host interactions are further complicated by the involvement of third actors in the system, such is the case with hyperparasitism, where parasites host their own parasites (Parratt & Laine, 2016). But

also host symbionts and especially the gut microbiome can play an important role in the evolutionary arms-race between parasite and host (Mukherjee et al. 2020). Parasites can create an alternative steady state of the gut microbiome or introduce specialised bacteria that could function as symbionts for the parasite rather than the host (White et al. 2018). Sometimes the gut microbiome can even protect a host from parasitic infections to some degree (Harris et al. 2019) or in reverse the parasite can protect the host from pathogenic prokaryotes (Ramanan et al. 2016). These parasite-mediated gut microbial changes can even occur on a within-species basis where the gut microbiome is dependent on the parasite species' genotype (Hahn et al. 2022).

Considering the role of symbionts and the dependence on a well-functioning host immune system for a persistent parasite, we focus here on a system including the cestode parasite *Anomotaenia brevis* and its intermediate host, the ant *Temnothorax nylanderi*. The peculiarity of this parasite-host interaction is that infection with the parasite extends the lifespan of the host several fold (Beros et al. 2021). This is likely in the advantage of the parasite in that it increases the chance of transfer to the definite, avian host (Plateaux, 1972). So in this case, the parasite needs its host to stay alive with a well-functioning immune system while evading attack and elimination by that immune system for years. We would therefore predict that the parasite should adopt a strategy to evade the host's immune system rather than suppress it. Indeed, there is some evidence for this, as parasites in hosts with a high parasite load seem to invest more in evading the immune system, as evidenced by their transcriptomes (Sistermans et al. 2023). Furthermore, a high parasite burden was also associated with stronger innate immunity in the ant host. However, it has never been studied whether the adaptive immune system of the host and the ability of the host to fight off bacterial threats is influenced by cestode infection, nor the exact immune pathways involved in cestode and bacterial immunity.

Another unknown is the role of the gut microbiome in this interaction. The immune system and the gut microbiome are often so intrinsically linked, that an immune response can cause alterations in the gut microbiome composition of an ant host (Negroni et al. 2020). In the case of *T. nylanderi*, the gut microbiome is known to be colony-specific but differs only slightly between different castes (Segers et al. 2019). Given evidence from other parasite-host systems and the intertwining of gut microbiome composition with host physiology (Leung et al. 2018), cestode infection may impact the gut bacterial community. This has been shown in other insects as well, for example in grain beetles where a tapeworm infection causes an increase in gut microbial diversity while decreasing the abundance of myobacteria (Fredensborg et al. 2020). Additionally, the gut microbiome seems to be linked to the ability of the cestode to establish itself successfully as parasite establishment was lower in beetles treated with antibiotics. In our system, however, this is difficult to study because *T. nylanderi* cannot be artificially infected with *A. brevis*. This means that it is unavoidable that infected ants have additional confounding differences from uninfected ants, as infected workers are fed much more frequently and are likely to be older than their uninfected nestmates (Beros et al. 2021; Scharf et al. 2012). Age and food intake may have an impact on microbiome composition (Klammsteiner et al. 2020; Su et al. 2022; Wang et al. 2019).

There is also the possibility that certain gut microbiota can increase the probability of cestode infection. Since *T. nylanderi* colony members largely share their gut microbiome composition (Segers et al. 2019), observed changes might be the cause of infection rather than them being initiated by the parasite. We

thus aim for analysis on how infection status is linked to microbiome composition. This also applies to differences in gene expression during bacterial infection, as the fitness costs of cestode infection are borne by the entire colony. Infected ants appear to bear little cost of parasite infection, as they are even more likely to be fertile after queen removal and have a longer lifespan (Beros et al. 2019; Beros et al. 2021), whereas uninfected nestmates of infected workers exhibit a higher mortality (Beros et al. 2021). Therefore, we suspect that *A. brevis* might influence the ability of a colony to fend off a bacterial infection as well.

In this study, we aim to investigate both transcriptional activity in the fat body, an important organ of immune defence, and the gut microbiome in response to cestode infection in *T. nylanderi* workers. To investigate the specificity of the immune response, we expose the haemolymph of our treated group to a sham bacterial immune challenge through bacterial lipopolysaccharide (LPS). LPS forms major outer surface membrane components of Gram-negative bacteria (Grabowicz et al. 2014), that shields the underlying peptidoglycan cell wall and insects typically respond to LPS injection with a strong immune response (Imler et al. 2000). In this study, we are therefore not only investigating the effects of cestode- or LPS-induced immune challenge, but also whether cestode infection weakens or strengthens the immune response to bacterial threats in a full factorial design. We will analyse transcriptional changes in the fat body to assess the specificity of the immune response and by 16S ribosomal DNA (rDNA) amplicon sequencing to assess the gut microbiome.

Material and methods

Colony collection and ant maintenance

We collected *T. nylanderi* colonies in February and June 2021 in the Lennebergwald close to Mainz, Germany (50.011605" N 8.174941" E). We used a total of 16 colonies for the analysis, 8 of which had *A. brevis* infected workers. The infected colonies contained a maximum of 30 infected workers and an average colony infection rate of 24% (Table S1). The ant colonies were relocated into slide nests consisting of a piece of Plexiglas with an oval recess and a piece of paper between two microscope slides covered by red foil (Stoldt et al. 2021). These slide nests were placed in the middle of a three-chambered plastered nest box also containing a tube filled with water. The ants were fed with a drop of honey and a leg of a cricket twice a week. The colonies were placed in a 20°C climate cabinet with a 12-hour day-night cycle. All colonies were given at least two weeks of acclimatization before experimental treatment.

LPS immune challenge experiment

We split up the 16 colonies into two cohorts. The first cohort consisted of ten colonies, five infected and five uninfected colonies. The second cohort consisted of six colonies, three of which contained infected ants (Table S1). We started the LPS immune challenge experiment on the 1st cohort end of June and for the 2nd cohort end of July 2021. We distinguished three types of ants: infected workers, uninfected workers from infected and uninfected colonies. For each type of ant, we conducted either one of two treatments: a) *LPS treatment*, for which we pricked an ant with a 10mm thick needle dipped in a 10

mg/ml solution of LPS dissolved in autoclaved 1% PBS, this needle was entered between the first and second segments of the abdomen pointed from the posterior towards the thorax. The needle was inserted deep enough to ensure that the LPS went directly into the haemolymph. b) *control treatment*, consisted of inserting a sterile needle between the first and the second segment of the abdomen without breaking through the cuticle. We did not cause a wound, as this could trigger an immune reaction. For each ant type, we took two ants per colony and treatment. After the treatment, we marked the ants with a red or green wire loop and placed them back into the colony. Both the order of the ants and the colour of the wire loop were randomised for all treatments.

About 24 hours (± 1 hour) after the LPS immune treatment, we dissected the ants in the same order in which they were treated. We removed the two marked ants from the nest and placed them in an arena. Then we cleaned our tools by dipping them in 70% ethanol and passing them through the flame of a Bunsen burner. We cleaned the utensils that were not fireproof with bleach, including our microscope glass, before pipetting 150 µl of autoclaved 1% PBS. For dissection, we placed the first ant in the PBS and decapitated her with forceps. We then dissected the abdomen by pulling the second segment from the first, usually separating the fat body and trachea in the first segment from the remains of the gaster. We then removed the trachea and placed the cuticle with the attached fat body in an Eppendorf tube containing 50 µl TRIzol. We then removed any cestodes, the crop and the hindgut from the midgut to place it in a second Eppendorf tube containing 10 µl of autoclaved PBS. We removed the hindgut, as it had already detached from the midgut in most dissections, making it impossible to invariably dissect both the midgut and the hindgut intact. After completion of a dissection, the tools and the microscope glass were cleaned as previously described and dissected the second ant. Immediately after each dissection, the fat body samples were placed on dry ice and the gut sample on ice. At the end of the day, the gut samples were placed in a -20 °C freezer with the fat body samples being stored at -80 °C. If a treated ant was dead on the dissection day, we repeated the treatment with another ant of that colony until we had enough samples from all colonies. On each dissection day, we conducted a dissection control, where we performed the dissection routine without ants by dipping our tools into the PBS on the microscope glass and dipping them into an Eppendorf tube filled with 10 µl PBS.

RNA/DNA isolation and sequencing

We selected seven infected and uninfected colonies as treatment or dissections from one infected and one uninfected colony had failed (Table S1 and S2). For RNA extraction, we homogenised the samples in TRIzol using an autoclaved plastic micropestil. Then we added 25 µl chloroform and centrifuged for 15 minutes. Then the upper aqueous phase was transferred to Eppendorf tubes containing 25 µl of 96% ethanol. RNA was isolated using the Qiagen RNeasy Mini extraction kit according to the specifications of the protocol (the same methods were used in Sistermans et al. 2023). After isolation, we sent the samples to Novogene (Cambridge, UK) for library preparation, quality control and sequencing. There, the mRNA was enriched with oligo(dT) beads and then randomly fragmented by adding fragmentation buffer. The cDNA was synthesised using an mRNA template and a random hexamer primer. A custom second-strand synthesis buffer (Illumina), dNTPs, RNase H and DNA polymerase I were then added to initiate second-strand synthesis. After a series of terminal repairs, A ligations and sequencing adapter

ligations, the cDNA libraries were completed by size selection and PCR enrichment. Illumina sequencing was performed after pooling according to the effective concentration of the libraries and the expected volume of data (Novogene Co., Ltd). Between 58 million and 67 million paired-end reads per sample were sequenced.

For DNA isolation of gut microbiome, we used the Masterpure DNA isolation kit with a lysozyme step for Gram-positive bacteria. We extracted DNA from the same samples as in the RNAseq experiment (Table S1 and S2). The extractions were performed under a clean hood that was irradiated with UV light for half an hour before isolation. In addition to the samples from the RNAseq experiment, we also isolated DNA from two dissection controls and from two empty Eppendorf tubes (hereafter referred to as the "DNA extraction control") to be able to assess contamination of our samples. After DNA isolation, samples were submitted to StarSeq (Mainz, Germany) for amplicon sequencing of the V4 region of the 16S ribosomal RNA gene. Amplification was performed using the primer combination 515F-806R. 300 bp paired-end reads were generated using Illumina MiSeq. De-multiplexing, adapter trimming and quality filtering were performed according to the usual Illumina MiSeq workflow of StarSeq. They also sequenced the two negative controls and one positive control in the form of the ZymoBIOMICS microbial community DNA standard.

Processing of RNA-seq reads

We employed FastQScreen v0.14.0 (Wingett & Andrews, 2018) to eliminate adapters and contaminating sequences originating from *A. brevis, Homo sapiens, Escherichia coli*, and vectors within the raw reads. Raw reads of 120 bp or longer were subjected to paired-end mode trimming using fastP (Chen et al. 2018), with a N-base cutoff of 15. We then used FastQC (Andrews, 2010) to evaluate read quality, which calculates the probability of base call errors. Filtered reads were then aligned to the *T. nylanderi* genome assembly (Jongepier et al. 2022) using HISAT2 v2.1.0 (Kim et al. 2015) with the --dta parameter, preparing for genome-guided assembly. We then sorted and indexed the resulting BAM files with Samtools v0.1.19 (Li et al. 2009) and utilized these to generate a genome-guided transcriptome assembly employing StringTie v1.3.6 (Pertea et al. 2015). Transcript sequences were extracted using gffread v0.11.4 from the merged GTF files (Pertea & Pertea, 2023). For gene abundance counting, we utilized the script prepDE.py retrieved from the StringTie webpage (Pertea et al. 2015). Then, the predicted amino acid sequences of the transcripts were obtained using TransDecoder v5.5.0 (Haas et al. 2013), followed by functional annotation using InterProScan v5.46-81.0 (Jones et al. 2014), where we obtained both the GO terms and Reactome terms for the reads. Finally, the KEGG IDs of the amino acid sequences were acquired using the online tool EGGNOG mapper 2.1.8 (Cantalapiedra et al. 2021), which provides insights into the network of gene products within KEGG pathways.

Differential gene expression analysis

We uploaded the gene count matrix for analysis in R. We performed two analyses on a subset of the whole dataset. First, we compared infected ants to uninfected ants from infected nests, and second, we compared ants from uninfected nests to uninfected ants from infected nests. We started by performing

PCAs on both data sets to locate possible outliers and to get a first idea of the main drivers of the data. For data analysis, we plotted the two subsets of the dataset against a negative binomial distribution with DESeq2 (Love et al. 2014). For the first infected colonies dataset, we ran DESeq2 thrice, once using a Likelihood Ratio Test, where we used the interaction between cestode infection and LPS immune challenge as the main factor, and using cestode infection, LPS immune challenge and colony identity as reduced factors. In the next two DESeq2 runs, cestode infection was used as the main factor with LPS immune challenge and colony identity as reduced factors or immune challenge as the main factor with cestode infection and colony identity as reduced factors, respectively. We entered the resulting differentially expressed genes (DEGs) from the three analyses into InterActiVenn (Heberle et al. 2015) in order to visualize the overlapping DEGs between the tests for cestode infection and LPS immune challenge. We then simulated 10,000 distributions by drawing a random sample from a list containing the same number of genes as our gene counting matrix. We checked whether the number of overlapping DEGs found could be random by creating a histogram of the random distributions and checking whether the actual number of overlaps was within this distribution. For the functional annotation of these genes, we divided them into up-regulated and down-regulated genes among the three factors (based on log2 fold change) and the overlapping genes and used the file we had created with InterProScan to run the DEGs through TopGO (Rahnenfuhrer, 2022) using a Fisher's exact test. We further investigated the DEG functions by running a KEGG enrichment analysis using our EGGNOG output and the ClusterProfiler R-package (Wu et al. 2021). Finally, we BLASTED our DEGs using diamond BLAST (Buchfink et al. 2014). We ran these BLAST terms through UniProt for *Caenorhabditis elegans* and *Drosophila melanogaster* for a more thorough understanding of the gene functions using a script from Stoldt et al. 2021. To determine whether functions related to immunity are enriched in cestode-infected ants or in LPS immune-challenged ants, we counted the number of BLAST/UniProt hits mentioning immunity in the up-regulated group in cestode-infected/immune-challenged ants and in the up-regulated group in uninfected/non-immune-challenged ants and tested their significance with a chi-square test. In the case of DEGs we would find for LPS immune challenge, we did one extra search through the BLAST and UniProt hits in order to find genes specifically involved in wound healing to test whether their involvement would confound the results in order to control for the fact that LPS immune challenged ants were also uniquely wounded by the LPS injection. Finally, we performed a Reactome enrichment analysis with the file obtained through InterProScan. Here, we created a count table for each set of differentially expressed genes (the DEGs for cestode infection and the DEGs for LPS immune challenge) and performed a Fisher's exact test for their Reactome terms against a universe of all Reactome terms and their counts. We then used the Benjamini-Hochberg method to adjust for multiple testing. We identified the functions of the Reactome IDs using the reactome.db package (Ligtenberg, 2019).

WGCNA analysis

We used the WGCNA R package (Langfelder & Horvath, 2008) in order to perform a Weighted Gene Co-expression Network Analysis (WGCNA). We first filtered out genes that have less than 10 counts for at least 5 samples. We then checked data for missing entries with a verbosity set to 3. For the network

construction steps we set a soft-thresholding power of 10 and the minimum module size to 100 based on visual confirmation through a TOM plot (figure S1). From this, we were able to identify 20 modules for which we performed GO enrichment and KEGG enrichment analysis in the same way as for DEG analysis. We then used the module eigengene values for each sample to build a linear mixed effects model using LPS immune challenge and cestode infection as predictors and colony identity as a random effect. We did this for each module and then tested the model for significance with an ANOVA, testing both fixed effects and the interaction between the two. We then corrected the p-values for multiple testing using the Benjamini-Hochberg method.

Data analysis of the gut microbiome

We checked the quality of the forward and reverse reads separately using the dada2 package on R (Callahan et al. 2016). Based on this quality control, we trimmed the forward and reverse reads at positions 220 and 180, respectively. After trimming, we de-noised the reads and removed chimeric reads, whereupon we derived amplicon sequence variants (ASVs). Using the ASVs, we filtered out mitochondria, chloroplasts and eukaryotes. The ASV table was further used to assess sequencing accuracy using the positive control, the ZymoBIOMICS Microbial Community DNA Standard. We detected eight sequences in the mock community that matched the eight expected reference sequences in approximately the same ratio (Figure S4). To account for cross-contamination during sequencing, we then used the prevalence method of the Decontam R package (Davis et al. 2018). For the downstream analysis we plotted the relative abundances using phyloseq (Mcmurdie & Holmes, 2013). We then decided to test for differences in alpha- and beta-diversity based on cestode infection, comparing the infected ants from the uninfected ants from infected colonies, including the LPS immune challenge as covariable. Then we repeated the analyses on the same samples investigating the effect of the LPS immune challenge, with cestode infection as a covariable and finally analysing whether we find an interaction between the two main factors: cestode infection and LPS immune challenge. Finally, we tested the whether infection or LPS immune challenge has an effect on any of the individual bacterial groups using the ANCOM-II package (Lin et al. 2022; Lin & Peddada, 2022) using the Benjamini-Hochberg method to adjust p-values for multiple testing. We then tested the effects of colony cestode infection on the alpha and beta diversity of the uninfected samples with LPS immune challenge as a covariate and for LPS immune challenge with colony infection as a covariate.

In addition, we analysed the differential gene expression in the three main identified groups based on beta diversity (Figure 3c) in the infected dataset (cestode-infected ants and uninfected ants in infected nests). We performed an LRT comparing the three groups considering cestode infection, immune challenge and colony identity. We examined the functions of the DEGs by BLASTing the DEGs with NCBI BLAST (Altschul et al. 1990).

Results

Differential gene expression analysis

The analysis of the infected colony data, in which we examined the consequences of individual cestode infection, the LPS immune challenge and their interactions, was based on a total of 16090 genes, of which 1764 showed differential expression. 1335 genes differed in expression between cestode-infected and uninfected workers, and 542 genes changed their expression due to the LPS immune challenge. These results were reflected in the PCA, where we observed that the first principal component, explaining 21% of the variance, separates samples with and without cestodes. The second principal component, which explains 13% of the variance, divides individuals with and without LPS immune challenge (Figure 1a). The expression of only three genes could be explained by the interaction between cestode infection and LPS immune challenge, suggesting that both immune stressors elicit largely independent responses in these ants. However, among the DEGs of the two main factors, we identified 115 genes whose expression was altered by both the LPS immune challenge and cestode infection (Figure 1b). By simulating random gene sets of 16090 genes with two sets of 1335 and 542 DEGs, we found a 95% confidence interval of 33 (lower percentile) to 57 (upper percentile) genes. Since our overlap is well above this with 115 overlapping genes, we have more DEGs in common than expected by chance (Figure S3). Looking at which direction these common genes changed their expression, we discovered that the different immune stressors had opposite effects on each of these 115 genes, i.e., if a gene was up-regulated in cestode-infected ants, it was found to be downregulated following the LPS-challenge and vice versa (see Figure S8 for a Venn diagram of directionality). For the functional annotation of the DEGs, we took three approaches: First, we undertook annotation using GO terms. Here, of the 1335 DEGs for cestode infection, we revealed 567 that were overexpressed in the uninfected group characterized by two GO terms, the first of which represents 33 genes involved in proteolysis (Figure 1d). Proteolysis has been shown to be involved in immune functions such as blood clotting and melanisation (Hoffmann, 1995). Among the 770 overexpressed DEGs in infected ants, we found translation with 21 representative genes, lipid metabolism with 20 representatives and proteolysis involved in protein catabolism with 12 representatives (Figure 1d). Although translation is a rather general and thus uninformative term, the upregulation of lipid metabolism suggests that infected ants, which are more often fed than other workers (Scharf et al. 2012), might have a higher need for long-term energy storage (Liu & Czaja, 2012). Furthermore, fat metabolism has an impact on the immune response of insects, as the fat body of insects is their most important immunological organ (Hoffmann & Reichhart, 2002). Proteolysis is part of protein degradation and might be used in cestode infected ants to degrade tapeworm proteins that are excreted by *A. brevis* into the ants' haemolymph and make up about 7% of all proteins in the haemolymph of these infected workers (Hartke et al. 2023). We also found glutathione metabolic process to be upregulated with six representative DEGs, this has been shown to be involved the activation of trained immunity (Ferreira et al. 2021).

For the 382 overexpressed DEGs of the not LPS-immune-challenged treatment, we found peptidoglycan catabolic process though these ants were not challenged by LPS. This could also be the result of these genes being involved in the downregulation of peptidoglycan catabolic process (Figure 1c), which has been shown to be involved in the breakdown of bacterial cell walls (Crump et al. 2021). Among the 167

genes down-regulated in the immune challenged workers, we again found lipid metabolic processes and glutathione metabolic processes (Figure 1c). This contradicts our expectations, as these processes are up-regulated in cestode-infected ants and therefore may suggest that these processes, both likely related to immune function, are regulated in opposite ways. However, this should be taken with caution as GO terms do not necessarily provide directional information about the underlying processes.

For the pathway analyses we took two approaches. First, we investigated KEGG IDs for which we found one hit in the group of cestode-infected ants, that hit being Coronavirus disease – COVID-19 with 14 representative genes. The COVID-19 pathway has been shown to be involved in the innate immune response (Ahmed-hassan et al. 2020; Tay et al. 2020) and the creation of oxidative stress (Amor & Blanco, 2020). The second approach was through reactome enrichment analysis through the Reactome IDs we obtained through InterProScan (Jones et al. 2014). Here we also only found significant terms for cestode infection, several of which were involved in vertebrate immunity and protein transport. As these were yet another confirmation of earlier results and as these results hold little more significance to this research we decided to put a more detailed description in supplementary paragraph 1.

Our final analysis compared the BLAST results of the DEGs for both cestode infection and LPS immune challenge, dividing the DEGs into five groups: upregulated in cestode-infected ants, downregulated in infected ants, upregulated in LPS immune challenged ants, downregulated in immune challenged ants and the overlapping genes. For first four groups of genes, we calculated the annotation rate of each of these groups and then counted the genes with UniProt terms related to immunity, stress, fertility, and longevity. We then compared the two treatment groups to test whether these proportions differed using chi-square tests (Figure 1e; 1f). Consistent with previous GO results we detect an inverse pattern in which GO terms of the downregulated DEGs of the cestode-infected group appeared among the terms of the upregulated DEGs from the immune challenged group (although most of them were not significantly enriched). We detected only two cases of significant overexpression, for fecundity and immunity in the cestode-infected group. For the immunity term, this seems to be somewhat consistent with our GO and reactome analysis, where antimicrobial immunity seems to be differentially regulated in general. In the BLAST results, this translates to up-regulated genes in the cestode-infected ants (36 genes) and the up-regulated genes in the uninfected ants (30 genes), where in both cases we find 20 genes specifically involved in the bacterial immune response. Among the down-regulated genes, we discovered antimicrobial peptides such as Toll receptor 3, which is involved in the immune response to Gram-negative bacteria and perhaps also to Gram-positive bacteria (Akhouayri et al. 2011). Furthermore we identified the two proteins 1-phosphatidylinositol 4,5-bisphosphate phosphodiesterase-like and dual oxidase 2, which are involved in the duox-dependent gut bacterial immune response (Ha et al. 2005; Lee et al. 2015) and peptidoglycan-recognition protein LC-like, which, true to its name, is involved in recognition of bacterial cell wall components (Choe et al. 2002). Dual oxidase 2 was also found to be differentially expressed in heavily cestode infected ants as opposed to lowly infected ants (Sistermans et al. 2023).

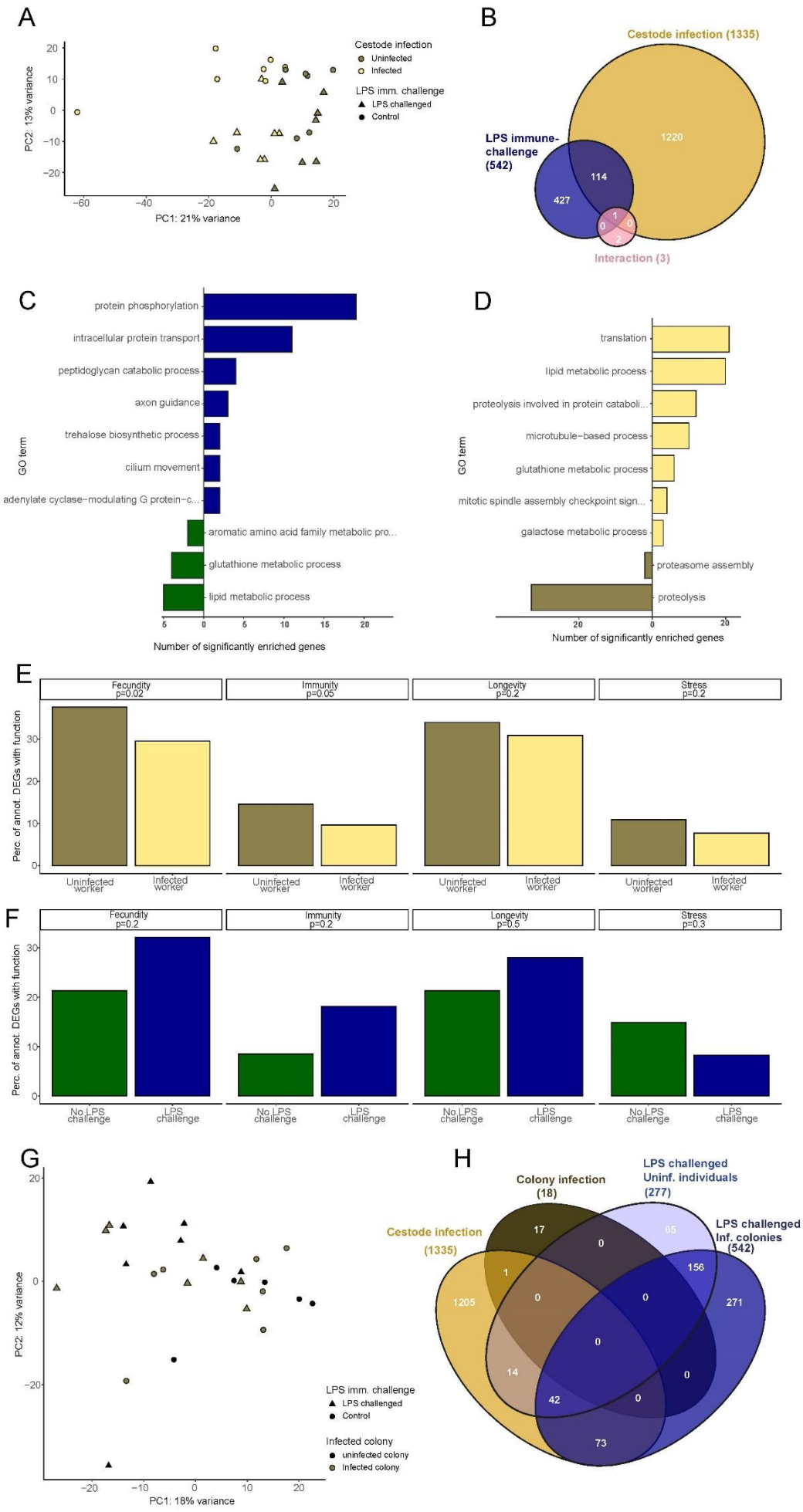

A
PC2: 13% variance
PC1: 21% variance
Cestode infection
Uninfected
Infected
LPS imm. challenge
LPS challenged
Control
B
Cestode infection (1335)
LPS immune-challenge (542)
1220
114
427
1
0
0
2
Interaction (3)
C
protein phosphorylation
intracellular protein transport
peptidoglycan catabolic process
axon guidance
trehalose biosynthetic process
cilium movement
adenylate cyclase−modulating G protein−c...
aromatic amino acid family metabolic pro...
glutathione metabolic process
lipid metabolic process
GO term
Number of significantly enriched genes
D
translation
lipid metabolic process
proteolysis involved in protein cataboli...
microtubule−based process
glutathione metabolic process
mitotic spindle assembly checkpoint sign...
galactose metabolic process
proteasome assembly
proteolysis
GO term
Number of significantly enriched genes
E
Fecundity p=0.02
Immunity p=0.05
Longevity p=0.2
Stress p=0.2
Perc. of annot. DEGs with function
Uninfected worker
Infected worker
F
Fecundity p=0.2
Immunity p=0.2
Longevity p=0.5
Stress p=0.3
Perc. of annot. DEGs with function
No LPS challenge
LPS challenge
G
PC2: 12% variance
PC1: 18% variance
LPS imm. challenge
LPS challenged
Control
Infected colony
uninfected colony
Infected colony
H
Colony infection (18)
LPS challenged Uninf. individuals (277)
Cestode infection (1335)
LPS challenged Inf. colonies (542)
17
0
65
1
156
1205
0
0
271
0
14
0
42
0
73

Figure 1 A. Principal component analysis of all samples from infected colonies, with colour indicating cestode infection status and shape indicating LPS immune response. PC1 and PC2 correspond to cestode infection status and LPS immune response, respectively. **B.** Venn diagram showing the number of DEGs in the immune challenge analysis, the cestode infection analysis and the interaction between immune challenge and the overlap between these three groups. **C.** Significant GO terms for the LPS immune challenge dataset, with the up-regulated terms in the immune challenged ants to the right of the Y-axis and the down-regulated terms in the immune challenged ants to the left of the Y-axis. **D.** Significant GO terms for the cestode-infected dataset, with the up-regulated terms in the cestode-infected ants to the right of the Y-axis and the down-regulated terms to the left of the Y-axis. **E.** Percentage of cestode-infected genes annotated by BLAST in terms related to fecundity, immunity, longevity and stress, and whether they are significantly enriched in either cestode-infected or non-infected ants. **F.** Percentage of genes annotated by BLAST with LPS immunity related to fecundity, immunity, longevity and stress with enrichment in LPS-infected or non-infected individuals. **G.** PCA of all samples not infected with cestodes from infected and non-infected colonies. **H.** Venn diagram of differentially expressed genes based on cestode infection and LPS immune challenge in both the infected versus uninfected ants dataset and the uninfected versus uninfected ants from uninfected colonies.

When studying the immune functions of the overlapping genes, which, as mentioned earlier, had differential directionality in expression in cestode infection and LPS challenge, we found a total of ten genes. Among these genes were two glutathione S-transferases, one defensin-like protein and three proteins involved in the toll pathway (these genes are plotted in figure S9). Glutathione S-transferase is involved in the detoxification response and the elimination of foreign substances (Hayes et al. 2005), defensin is involved in the antibacterial immune response in insects (Dimarcq et al. 1994) and the toll pathway, in this case represented by two toll-like receptors and protein spaetzle, forms a signalling cascade specifically in the respiratory epithelium to prevent the overexpression of antimicrobial peptides (Akhouayri et al. 2011). Examining the GO terms of the overlapping genes, we again found the glutathione metabolic process (Figure S6) to be one of the terms we uncovered on opposite sides of expression in the GO terms for both individual factors.

In our transcriptome analyses of uninfected ants both from uninfected and infected colonies, we did not detect a clear clustering by colony infection status or LPS immune challenge in the PCA (Figure 1g). We identified 18 DEGs responding to colony infection, eight of which were downregulated and ten were upregulated. We found a single GO term of DNA integration with three representative genes downregulated in ants from infected colonies. DNA integration is involved in transposition (Khoury et al. 2018) and DNA repair (Kselíková et al. 2022) and could possibly be an indication of stress as uninfected ants in infected colonies show a higher mortality (Beros et al. 2021). For the LPS challenge we found 277 differentially expressed genes, 70 downregulated and 207 upregulated. When we compared these genes to the LPS immune-challenged DEGs in the infected dataset we found 156 overlapping genes, all with the same in their directionality (figure 1h). It should be noted that in both analyses the uninfected workers from infected colonies were involved, which might explain the similarity of the pattern. When investigating functions within this dataset we only found upregulated terms (figure S7). We reveal some coherence with the dataset containing infected ants such as

peptidoglycan metabolic process and a term closely related to lipid metabolic process: fatty acid metabolic process. We found no significantly enriched KEGG terms and no significantly enriched BLAST terms for either analyses.

WGCNA analysis

We identified 20 co-expression modules, spanning between 165 genes (royal blue, Figure 2a) and 2051 genes (turquoise). Consistent with our expression analysis, we found the highest co-expression level with cestode infection with a total of eight modules, while four modules were co-expressed with LPS immune challenge. In terms of interaction, we discovered no link to our modules (Figure 2a). As for the overlap of co-expression, we found the modules related to both cestode infection and LPS immune challenge, with the correlation two of these modules exactly reversed. Since we constructed a signed network in WGCNA, this means that the eigengene values can be directly translated into a positive correlation and therefore the direction of expression within these modules can be considered opposite. This is consistent with the patterns of overlapping DEGs, which were also mostly opposite. The notable exception is the magenta module, where cestode infection and LPS immune challenge link to in the same direction.

This brings us to the functions of these modules, starting with magenta, where we find in KEGG enrichment mainly metabolism related pathways represented by metabolic pathways, biosynthesis of secondary metabolites, microbial metabolism in different environments and carbon metabolism (Figure 2e). Examining the role of DEGs in this context, it appears that genes are upregulated in both LPS-challenged ants and cestode-infected ants (Figure 2c), although given that none of the DEGs in these two groups overlap, they are likely to be different genes with similar functions. These metabolic pathways involve very basic metabolic functions such as glycolysis, the citrate cycle and the glyoxylate cycle (Nishizuka, 1980), despite the name of microbial metabolism in diverse environments this does not seem to be linked to the immune system (Nishizuka, 1980). We find the same indication with the singular representative GO term in the magenta module with glycolytic process. One explanation for the similar directionality of metabolic genes in response to cestode infection and immune challenge could be that both lead to stress, which are common following bacterial infection (Wickersham et al. 2017) and cestode infection (Cancela et al. 2019). In the other two modules which co-express with cestode infection and LPS immune challenge we find no enriched GO terms or KEGG terms, with the exception of the cyan module which is co-expressed for cestode infection and LPS challenge in the uninfected dataset. One exceptional module of interest is the brown one, which is strongly linked with the LPS immune challenge (p=4.87e-07) and lightly with cestode infection (p=0.044). What is of note in this module is the large number of upregulated genes in the LPS challenged group, namely 313 out of 382 DEGs (figure 2c). Considering LPS does not generally cause side-effects (Zielen et al. 2015) and most of its effects are that of an altered immune response or inflammation (Alves et al. 2021; Korner & Schmid-Hempel, 2004) the brown module likely mainly consists of genes involved in the immune response, including adaptive and innate. For the modules that are only co-expressed with cestode infection we find multiple neurogenerative diseases in the purple module (figure 2c), with Parkinson's disease, Huntington disease and prion disease. These pathways seem to be especially involved in lipid

metabolism in insects (Ambegaokar et al. 2010; Fortini & Bonini, 2000; Z. Liu & Huang, 2013) and suggests that cestode-infected ants have an altered lipid metabolism and/or an altered immune response. In the light cyan module we once again uncover the coronavirus pathway which, as mentioned earlier is involved in the innate immune response (Ahmed-Hassan et al. 2020). Further the turquoise module linked to cestode infection showed the enriched Go Terms G protein-coupled receptor signalling pathway, DNA integration and ion transmembrane transport. Genes in this module might be involved in the communication between cestode and parasite considering both G-protein coupled receptors and ion transmembrane transport are involved in cellular signal transmission (Gamper & Shapiro, 2007; Seifert & Wenzel-Seifert, 2002). Furthermore, similar signalling pathways have been previously found within this system such as protein import into the nucleus in the host and sodium ion transport in the parasite (Sistermans et al. 2023). Finally, in the black module biosynthetic process was significantly enriched, which is again a general process and difficult to interpret in the context of cestode infection.

When investigating the uninfected dataset, we found no significant co-expression with colony infection status but five modules significantly linked to the LPS immune challenge. Three of these modules also co-expressed with LPS immune challenge in the infected dataset whereas two, pink and cyan are not. Here we would like to highlight the function of the cyan module, which co-expresses with cestode infection in the infected dataset and LPS immune challenge in the uninfected dataset (figure 2f). A single GO term L-phenylalanine catabolic process was enriched, which seems to have connections to melanisation in insects (Parthasarathy et al. 2018).

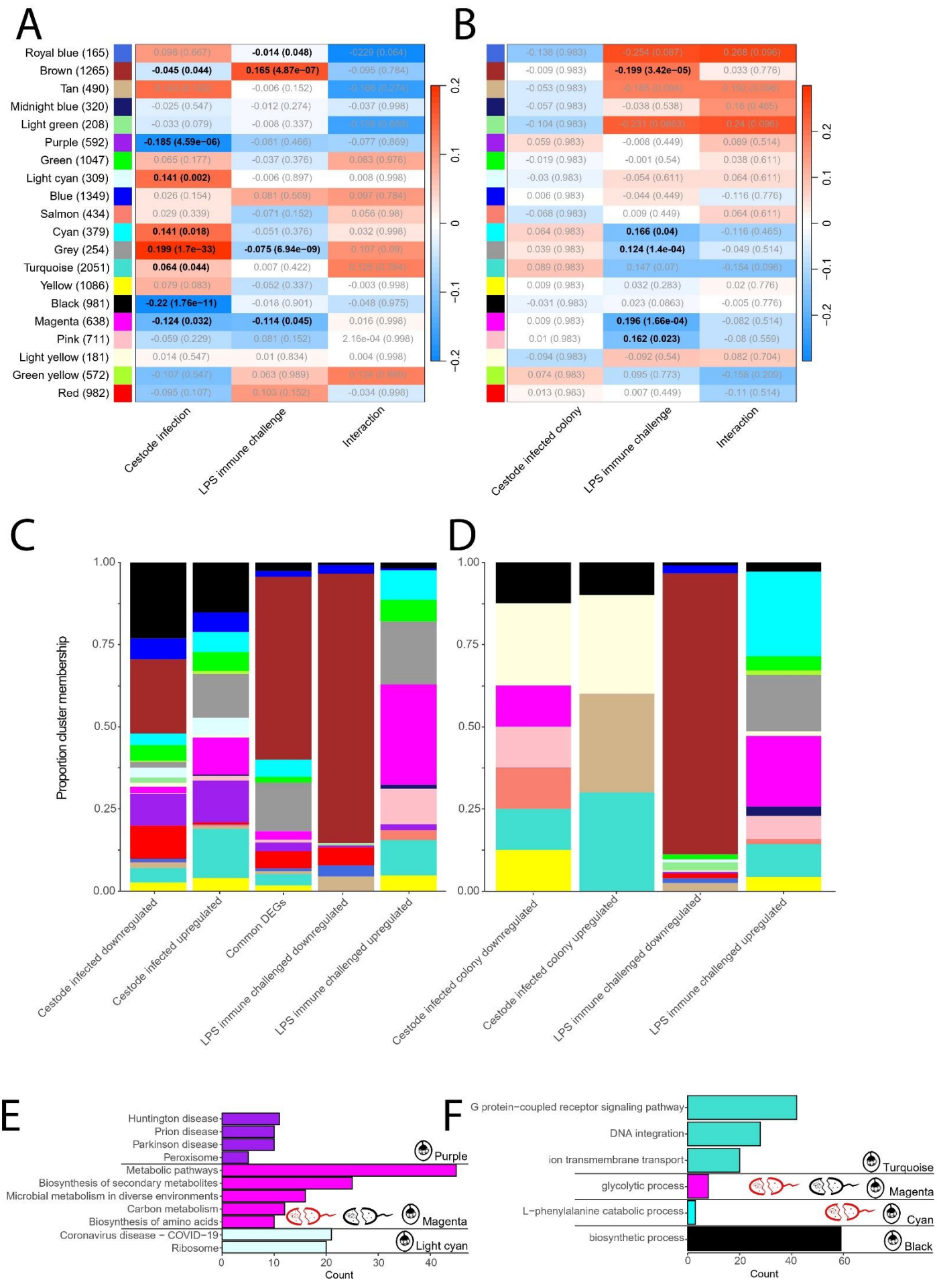
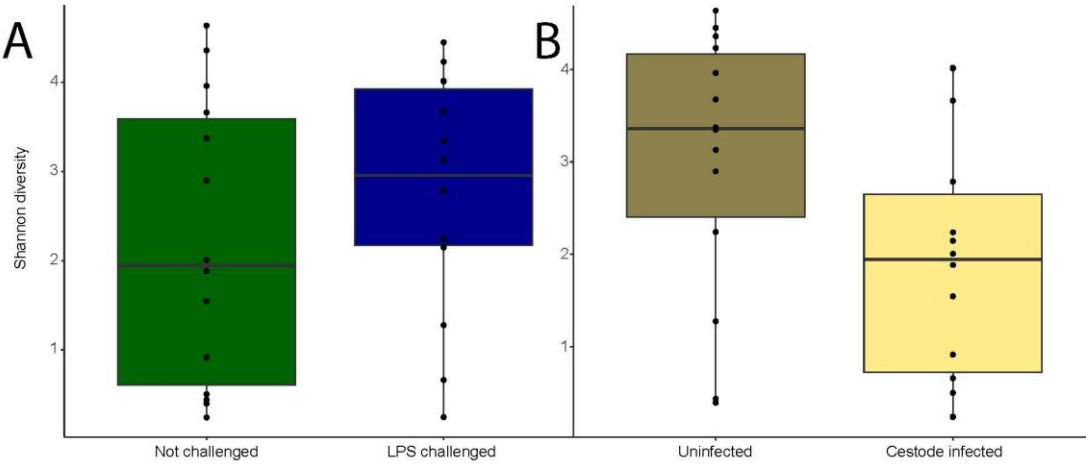

A
Royal blue (165)
Brown (1265)
Tan (490)
Midnight blue (320)
Light green (208)
Purple (592)
Green (1047)
Light cyan (309)
Blue (1349)
Salmon (434)
Cyan (379)
Grey (254)
Turquoise (2051)
Yellow (1086)
Black (981)
Magenta (638)
Pink (711)
Light yellow (181)
Green yellow (572)
Red (982)
Cestode infection
LPS immune challenge
Interaction
B
Cestode infected colony
LPS immune challenge
Interaction
C
Proportion cluster membership
Cestode infected downregulated
Cestode infected upregulated
Common DEGs
LPS immune challenged downregulated
LPS immune challenged upregulated
D
Cestode infected colony downregulated
Cestode infected colony upregulated
LPS immune challenged downregulated
LPS immune challenged upregulated
E
Huntington disease
Prion disease
Parkinson disease
Peroxisome
Metabolic pathways
Biosynthesis of secondary metabolites
Microbial metabolism in diverse environments
Carbon metabolism
Biosynthesis of amino acids
Coronavirus disease - COVID-19
Ribosome
Purple
Magenta
Light cyan
Count
F
G protein−coupled receptor signaling pathway
DNA integration
ion transmembrane transport
glycolytic process
L−phenylalanine catabolic process
biosynthetic process
Turquoise
Magenta
Cyan
Black
Count

Figure 2 Eigengene correlation heatmaps of all WGCNA modules for the **A.** individual-level and **B.** colony-level cestode infection data sets. Each module is marked with a colour and the number of genes per module is indicated in brackets. In each cell of the heatmap, the correlation coefficient and the adjusted p-value are given in parentheses. Module representation among DEGs from the DeSeq2 analyses for the **C.** individual-level and **D.** colony-level cestode infection data sets. Bar chart of the **E.** KEGG- and **F**. GO-enriched terms for all modules with $p < 0.10$. The significantly co-expressed modules were indicated with a broken bacteria pictogram (for LPS challenge) or a cestode pictogram (for cestode infection) next to the colour names in the enrichment barplots. A red bacterium pictogram indicated significant co-expression in the uninfected ants dataset.

Gut microbiome analysis

Comparison of cestode-infected and uninfected workers, both from infected colonies, revealed lower gut microbiome alpha diversity in cestode-infected workers (GLMER p=0.03; Fig. 3b). No changes in microbial alpha diversity were detected in response to the LPS immune response (Fig. 3a, LME p=0.36). When examining beta diversity, we found a strong separation between cestode-infected samples, uninfected samples, and uninfected samples from uninfected nests with a bootstrap confidence of 100 (Fig. 3c). Cestode-infected ants were predominantly represented in a group dominated by Entomoplasmatales (8 out of 12 samples), uninfected ants fell into a group with a microbiome dominated by *Wolbachia* (8 out of 16 samples) and ants from uninfected colonies mainly represented a group whose most common taxon was Pseudomonodales (8 out of 14 samples). Testing for Bray-Curtis dissimilarity in this dataset, we also find that beta diversity was reduced in infected workers (PERMANOVA; p=0.004), while LPS immune challenge (PERMANOVA; p=0.283) and interaction (PERMANOVA; p=0.762) had no effects. However, when we analysed whether any ASVs were overrepresented in any of these groups using ANCOM-II (Lin et al. 2022; Lin & Peddada, 2022) we found no overrepresentation of any taxon, neither upon cestode infection nor LPS immune challenge. We could therefore not statistically pin the patterns observed to specific taxa.

In addition, we performed a differential gene expression analysis for this dataset based on the three main groups shown in Figure 3c. We revealed four differentially expressed genes and therefore conclude that microbiome composition types are not linked to gene expression in the fat body. One of these genes, MSTRG.12040 was also overexpressed in LPS immune challenged ants from the infected dataset, and another one, MSTRG.18320 was downregulated in infected ants. The other two were present only in this dataset. When we examined the functions of these genes, we found a facilitated trehalose transporter responsible for the uptake of trehalose into the fat body (Thompson, 2003) and phenol oxidase 1 which is involved in the innate immune reaction, reacting to bacterial LPS (Kim et al. 2002). As for the genes that were co-differentially expressed, we only found a significant match with the gene co-differentially expressed with LPS immune challenge. The highest BLAST hit was with probable cytochrome P450 which is involved in insect homeostatic metabolism (Dermauw et al. 2020).


A
B
Shannon diversity
1
2
3
4
Not challenged
LPS challenged
Uninfected
Cestode infected


C
Entomoplasmaceae/Entomoplasma
Anaplasmataceae/Wolbachia
Pseudomonadaceae/Pseudomonas
Anaplasmataceae/Wolbachia
Staphylococcaceae/Staphylococcus
Moraxellaceae/Acinetobacter
Streptococcaceae/Streptococcus
Rizobiaceae/Aminobacter
Weeksellaceae/Cloacibacterium
Pseudomonadaceae/Pseudomonas
Other
LPS immune challenge
LPS challenged
Not challenged
Cestode infection
Uninfected nest
Infected nest, uninfected
Infected nest, cestode infected
0.00
0.25
0.50
0.75
1.00
Abundance

Figure 3 Shannon diversity of the gut microbiome compared between **A.** immune challenged and not immune challenged (p=0.36) and **B.** cestode infected vs uninfected (p=0.03). **C.** Beta diversity dendrogram of all samples, the colour within the abundance bar plots indicating the bacterial groups, the shape of the tree endings indicating the LPS immune challenge and colour of the shapes the cestode infection status.

Discussion

Our study investigated whether two types of immune challenges, cestode infection and injury coupled with LPS exposure, have interactive or independent effects on fat body transcriptional activity and gut microbiome composition in ants. In both analyses, we found no evidence for an interaction and stronger consequences with the persistent tapeworm infection, which is already established in the larval stage of ants, then with the one-time LPS immune challenge. Despite a lack of an interaction, we found more genes than expected by chance that changed their expression in response to both immune stressors. Not only that, these genes showed opposite effects, being upregulated following the short-term sham bacterial challenge, but were less expressed in cestode-infected workers. We also found shifts in the gut microbiome, in particular a loss of microbial diversity in cestode-infected ants. Here, three groups were graphically apparent, with healthy workers from infected colonies often showing *Wolbachia*-dominated communities, cestode-infected workers harbouring largely Pseudomonodales and uninfected workers from uninfected colonies exhibiting highly diverse communities.

Cestode infection suppresses immune function of host.

T. nylanderi workers infected with *A. brevis* cysticercoids in the haemolymph showed differential expression of 1300 genes, whereas the single immune response with LPS resulted in activity changes of only about 500 genes in the fat body. Despite this large number of DEGs, we find that the expression of only three genes depends on the interaction between these two immune stressors. Tapeworm-infected workers thus respond to the LPS immune challenge similarly to healthy workers, except for these three genes, which unfortunately did not result in any BLAST hits. Two of these interaction DEGs are located in the brown WGCNA cluster, which was linked to both immune stressors, but had no enriched GO or KEGG terms. To determine the exact functions of these two genes, future research should focus on the silencing these genes and investigate their role in the immune response or host metabolism. A more surprising finding that undoubtedly indicates an impact of *A. brevis* on the immune response is the direction of expression of genes that are co-differentially expressed in both LPS-immune challenged and cestode-infected workers. Here we discovered that genes that are upregulated in cestode-infected ants are downregulated in LPS-immunised individuals and vice versa, without exception. Genes that are differentially expressed following the LPS challenge are likely to be involved in immunity (Zielen et al. 2015) and in response to injury, and there again wound-healing can be seen as part of an immune response (Johnston & Rolff, 2013; Krautz et al. 2014; Tsirogianni et al. 2006). These opposite shifts in expression could thus indicate that some immune pathways are down-regulated in cestode-infected

ants. Indeed, we also found several GO terms based on the DEG analysis that pointed in opposite directions, in particular two immune functions with glutathione metabolic process and lipid metabolic process. Finally, two of the co-expressed WGCNA modules were linked to LPS challenge and cestode infection in opposite directions. Therefore, infection with *A. brevis* appears to suppress the immune system of *T. nylanderi* to some extent, but the system still remains inducible and can be activated following a (sham) bacterial exposure. Somewhat unexpectedly, however, the overall immune response is somewhat impaired, given the large negative overlap between LPS-challenged and cestode-infected workers. This is new, as studies have shown that parasites can have an immunosuppressive effect on their host (Colinet et al. 2010) including cestodes (Vendelova et al. 2015), but little research has been done in the host's ability to fight of other pathogens despite this immune suppression.

The brown module does not only contain many immune genes, but the GO enrichment analysis points to the importance of the glutathione metabolic process, which seems to be mainly involved in protection against severe oxidative stress (Maher, 2005) and detoxification (Ranson & Hemingway, 2005). Glutathione metabolic process is found to be oppositely altered with the LPS immune challenge (positive) and the cestode infection (negative) based on three genes that are differentially affected in their activity by these two immune stressors. The cestode infection could cause an increase in the glutathione response, as this has already been observed in another cestode-arthropod system (Sánchez et al. 2016), where shrimps were better able to deal with toxin exposure when infected by a cestode. This would suggest that in times of bacterial immune stress, *T. nylanderi* should deal less with oxidative stress, probably hinting at the creation of a hypoxic environment to weed out bacteria in the haemolymph (Thompson et al. 2017). This would be a general immune response, and the increased oxidative stress response is likely to make the host less able to deal with an immune challenge. A possible solution could be that the parasite itself contributes to the immune response, as proteins related to immunity have been found to be released by *A. brevis* into *T. nylanderi* (Hartke et al. 2023).

Another indication of how the immune response differs with tapeworm infection is the cyan module where we found both a GO term L-phenylalanine catabolism enriched and a correlation in the opposite direction for immune challenge and cestode infection (Parthasarathy et al. 2018). Melanisation is involved both in the formation of the cuticle as well as the general immune response where insects are capable of encapsulating pathogens (Luz et al. 2022). Ants infected with cestodes do show an opposite trait expression, as they have a less melanized and sclerotized cuticle, and melanised cestode larvae have never been found in the haemolymph of infected ants so far (pers. obv. based on ± 1000 dissections). Therefore, it could be that the cestode suppresses the melanisation response even in the presence of a bacterial threat and thus we do not find significant co-expression upon LPS challenge in the infected dataset. This would imply that we should find an interaction, alas we did not.

However, several questions remain, the most pressing being how bacterial immune challenge affects the parasite. In this system, it has already been shown that *A. brevis* also has some ways to evade the immune response (Sistermans et al. 2023) and in conjunction with these data, we suggest that *A. brevis* strikes a delicate balance between immunosuppression and evasion of the immune system. We propose a follow-up experiment to investigate *A. brevis* gene expression in response to LPS challenge. Another open question is the specific immune pathways that *A. brevis* suppresses. The brown WGCNA cluster

appears to contain genes involved in the general host immune response. We propose that specifically the hub genes within this cluster be further investigated by RNAi injection to determine the specificity of the immune response and immunosuppression.

Cestode-induced transcriptomic changes

Aside from the change in immune function, we found several transcriptomic changes in *T. nylanderi* in response to *A. brevis* infection. In accordance with previous studies (Hartke et al. 2023; Sistermans et al. 2023), we identified several transport functions to be co-expressed with cestode infection in the turquoise WGCNA module with G protein-coupled receptor signalling pathway and ion transmembrane transport (Gamper & Shapiro, 2007; Seifert & Wenzel-Seifert, 2002). G protein-coupled receptor signalling pathway is also strongly involved in cestode development (Paludo et al. 2020). As this is an important pathway for cestodes especially, they might use it also to communicate with their host considering the involved genes are very well-conserved throughout animal evolution (Broeck, 2001). For insects, G protein-coupled receptor functions seem to be extremely diverse and can be involved in cuticle development (Mendive et al. 2005), fecundity (Jiang et al. 2013), stress response (Terhzaz et al. 2012) and ion and fluid transport (Kwon et al. 2012) among others (Liu et al. 2021). Furthermore, we found a possible cluster of immune genes was found with the light cyan cluster which co-expressed with cestode infection. This module was enriched for the COVID-19 KEGG pathway, which seems to be involved in the innate immune response, especially inflammation (Tay et al. 2020). This pathway was also the sole significantly enriched pathway for the DEGs upon cestode infection. Considering it only co-expressed with cestode infection, the possibility exists that this inflammation response is specific to animal parasites as LPS challenges have caused an inflammation response in *T. nylanderi* (Zielen et al. 2015).

No conclusive evidence of gut microbial intervention

Finally, when analysing the gut microbiome, we found a reduction in Shannon diversity and a difference in Bray-Curtis dissimilarity exclusively in cestode-infected ants. Unexpectedly however, the LPS challenge did not cause a reduction in gut bacterial diversity as was found in the congeneric species, *Temnothorax rugatulus*, where Shannon diversity of the microbiome was reduced after a similar sham bacterial challenge (Negroni et al. 2020). There are a few explanations for this difference, the most straightforward one being that the last common ancestor between the two species lived in the Oligocene (Prebus, 2017) and therefore both species could have developed different immune responses against bacterial threats. Another explanation might be the fact that *T. rugatulus* occupies an entirely different niche than *T. nylanderi* does, namely pine forests at high elevation rather than temperate forest. This implies a different diet and high elevation species and populations have been shown to have a different microbiome (Zhao et al. 2018) in the first place and their immune systems might not be adapted to the bacterial LPS that these ants were treated with, thus necessitating a more general immune response at the cost of gut bacteria. Finally, it could be that certain gut microbiota would mediate the immune response (reviewed by Round & Mazmanian, 2009) in a similar manner that

cestodes do and that these gut microbiota are not present in *T. rugatulus*. This is perhaps best exemplified by the relative dominance of *Wolbachia* in our dataset, which is entirely absent in *T. rugatulus*. *Wolbachia* does actually seem to have some immune regulating properties when present in a host as non-pathogen in that it's capable of mediating the production of reactive oxygen species (Zug & Hammerstein, 2015). In the case of a parasite-induced decrease of Shannon diversity our results mirror previously found results where a decrease in gut microbial diversity was found upon parasite infection in firebugs (Onchuru et al. 2018). There are also several explanations for this reduction, firstly it could be the result of a previous immune response to *A. brevis* which resulted in only intracellular bacteria remaining. This would somewhat explain the relative dominance of *Entomoplasma* and *Wolbachia* in the infected groups (12 out of 14 samples had one or more intracellular bacteria as the largest group). Intracellular bacteria are considered more difficult to eradicate by the innate immune system and demand a specialized immune response (Munang'andu, 2018; Thakur et al. 2019). A second explanation for the cestode-mediated decrease of Shannon diversity would be because of differences in age and diet, which have been shown to influence the gut microbiome (Klammsteiner et al. 2020; Su et al. 2022; Wang et al. 2019). Firstly, due to the fact that cestode infected ants live up to five times longer than uninfected ants (Beros et al. 2021), there was a higher chance that infected workers in this experiment were older than uninfected workers. Worker age cannot be controlled for as we are unable to infect ant larvae with *A. brevis*. However, since younger colonies are generally smaller than older colonies, infected workers from smaller colonies have a higher chance of being younger. In one of these colonies, which had 16 workers, we observed infected and uninfected workers with similar gut microbiomes. This is of course just a single small colony and does not serve as a strong argument for this point, therefore further research efforts should take into account worker age, possibly through artificial infections by feeding ants with infected bird faeces or by inducing the final life stage of the cestode on a culture, which has been shown to be possible with other cestode species (Smyth, 1946; Wedekind et al. 1998; Weinreich et al. 2014). As for diet, this is also likely to be different in infected ant uninfected workers, since infected workers depend on their uninfected nestmates to be fed (Beros et al. 2015). We did control for this as much as possible, taking only nurses close to the brood and the infected workers rather than foragers outside of the slide nests. These workers have been shown to have similar metabolism (Beros et al. 2021) and likely have similar diets due to them depending on the foragers' feeding as well (Behmer, 2009). Furthermore, colonies were given a very invariable diet of cricket legs and honey. Therefore we do not believe that diet should have played a large role in creating differences between infected and uninfected workers.

Finally we found no specific bacterial groups to be dominant in any of our treatments. In cestode-infected ants this could be the result of an immune reaction upon cestode infection as larvae which we discussed earlier. This is not unheard of as immune challenges in larval stages of insects have shown to have profound effects on the adult gut microbiome and the microbiome during metamorphosis (Critchlow et al. 2019; Krams et al. 2017). If that is the case, we wouldn't necessarily find different groups of bacteria to be associated with infection but rather groups of bacteria that are resistant to certain immune reactions, as we found multiple taxa of intracellular bacteria like *Wolbachia* and *Entomoplasma*, which do indeed require different immune reactions (Reece & Kaufmann, 2019). Another explanation would simply be that individuals within a colony would largely display a similar gut

microbiome, as has been found earlier with *T. nylanderi* (Segers et al. 2019). Since this study also linked the gut microbiome to productivity, it would be advisable to design future experiments where the productivity of infected colonies is also taken into account. This would not mean that the cestode plays a part in this system since we also do not find any differences between infected colonies and uninfected colonies. Whether this change does indeed create a different, but non-specific gut microbiome in infected ants also needs more investigation. What makes this particularly of interest within the system is the influence on development that the gut microbiome can have on their host (Coon et al. 2014). Disturbances in the gut microbiome could therefore also lead to abnormal cuticle development, this has been shown in herbivorous turtle ant, whose gut microbiome is essential for normal cuticle development (Duplais et al. 2021). It is therefore not unlikely that even a disturbance in the gut microbiome, rather than a targeted change in the gut microbiome could lead to abnormal development, something very visible in the phenotype of infected *T. nylanderi* (Trabalon et al. 2000).

Conclusion

We find strong evidence in the transcriptome data of an immunosuppression in cestode-infected ants. Nevertheless, the inducible immune response of cestode infected workers was comparable to uninfected workers so that we did not detect an interaction between the two immune challenges. Thus, we show that the complex co-interaction between parasite and host has led to the parasite's intimate understanding of its host's immune system at the molecular level. Therefore, this parasite developed mechanisms to both protect themselves from the host and from other microbial threats as it walks the thin balance between immune manipulation and avoidance.

Acknowledgements

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Supplementary materials

Reactome pathway analysis

Our final pathway enrichment analysis approach was through the Reactome IDs we obtained through InterProScan (Jones et al. 2014). For this analysis we did not find any significant enrichment for the LPS immune challenged DEGs but we did we document a total of 164 terms for the cestode infected DEGs. The terms with the largest number of representatives can be seen in figure S5. The highest representative term with over 600 genes is neutrophil degranulation, which is involved in the antimicrobial response and inhibition of neutrophil degranulation can promote bacterial survival by preventing the targeting of antimicrobial proteins through the phagosome and plasma membrane (Eichelberger & Goldman, 2020). Additionally insect haemocytes and mammalian neutrophiles are sensitive to the same inhibitors (Browne et al. 2013), so that the presence of pathway terms related to neutrophil degranulation could indicate a differential immune function. The second most enriched term was peroxisomal protein import with over 300 representative genes, which has been described as a general protein import pathway (Baker et al. 2016). This lines up with a previous transcriptome study on *T. nylanderi*, which demonstrated that cestode-infected ants have an altered protein export function (Sistermans et al. 2023). In other parasite-host systems this function was also found to be changed with infection (Berger et al. 2021).

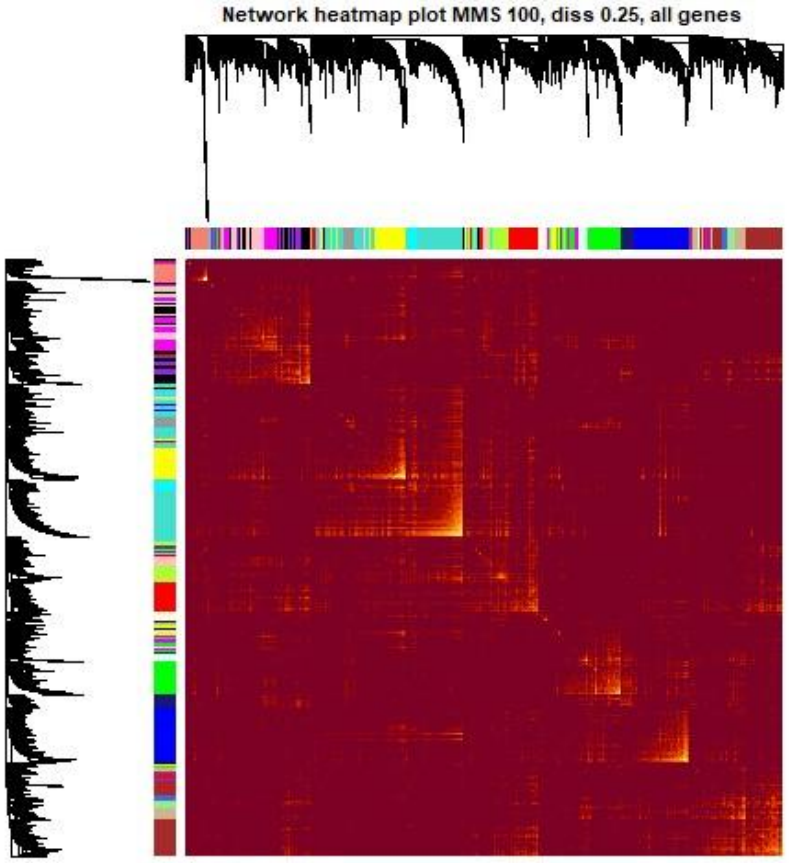

Figure S1 TOM plot with a minimum module size of 100, where the lighter colour in the heat map corresponds to stronger co-expression and the different colours to the modules constructed by WGCNA. Based on this TOM plot we decided to go for a module size of 100 where several modules (e.g. tan, turquoise, yellow, red green, blue, brown) clearly overlap to with the high co-expression in the heat map.

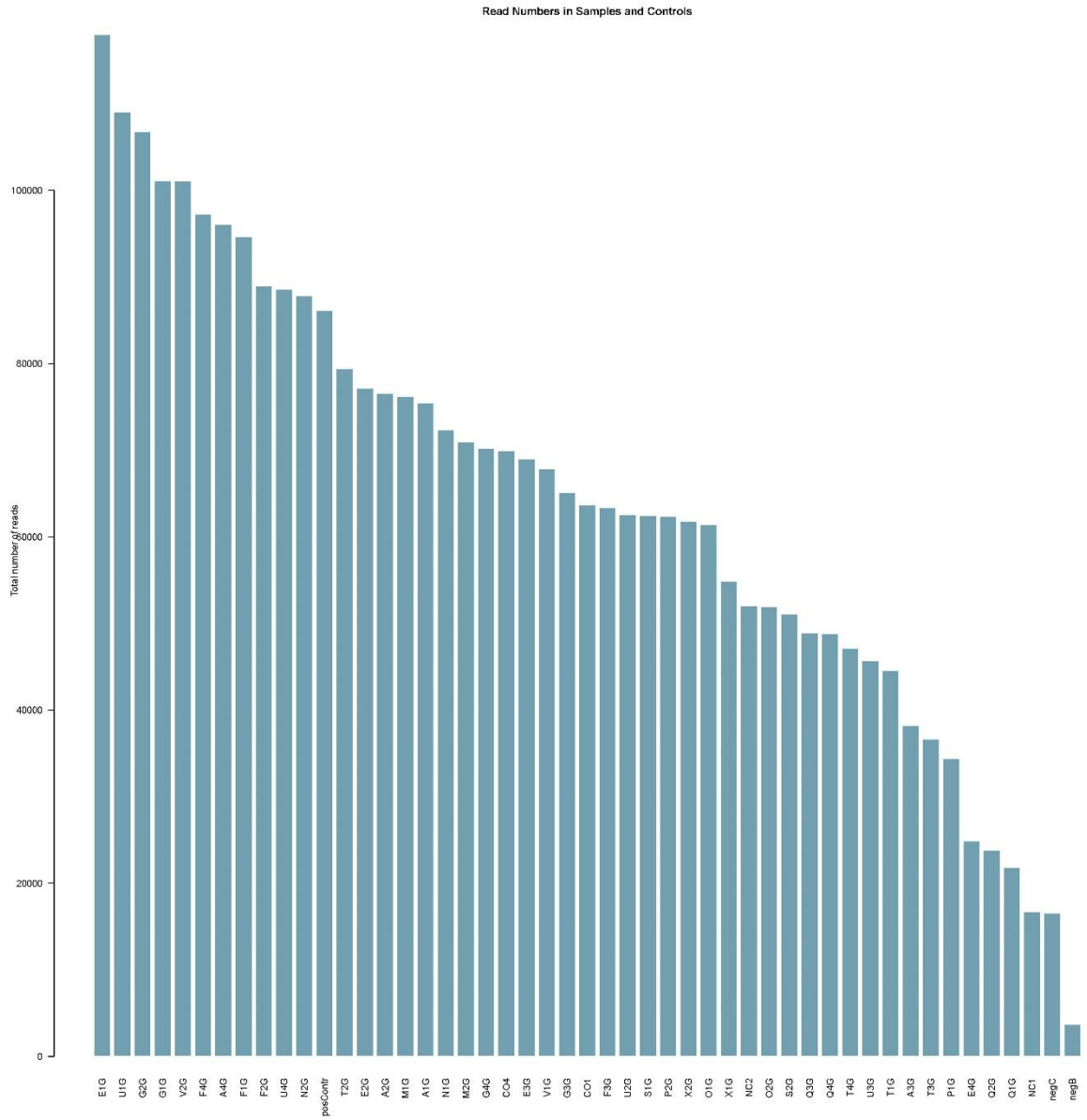

Figure S2 Total number of reads plotted per sample. It can be seen that the negative controls (negC and negB) have the lowest representation in read number, meaning relatively low cross-contamination during the sequencing.

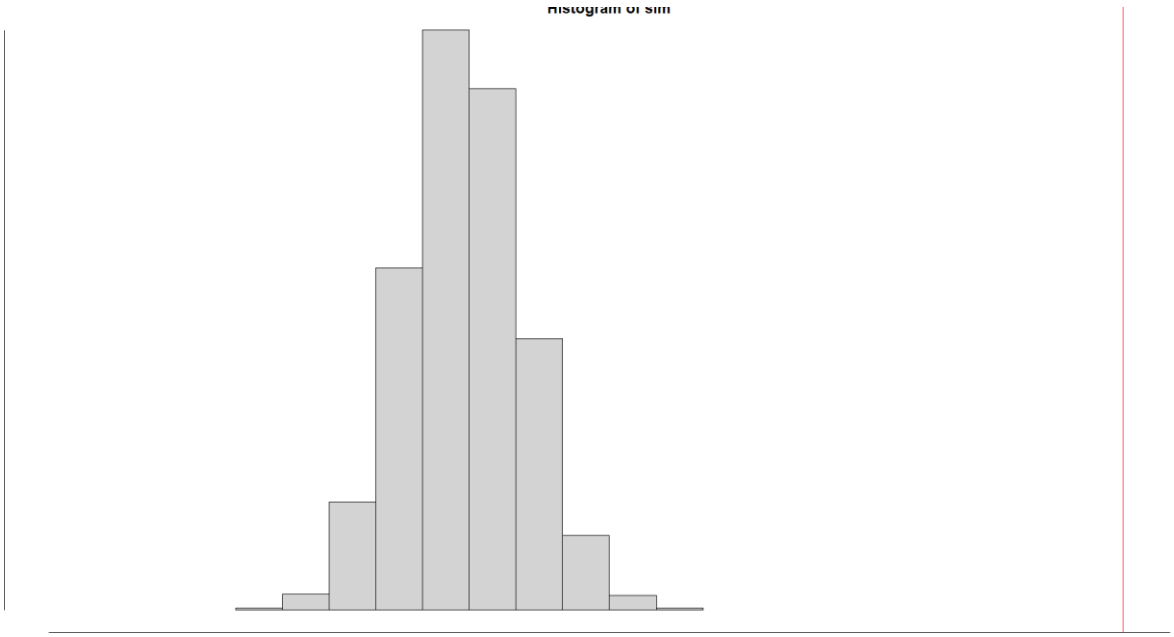

Figure S3 Histogram of the overlap of DEGs in 10000 simulations of a dataset of 16090 genes with two sets of 1335 and 542 DEGs, 95% of the cases can be found between 33 and 57 overlapping DEGs, the red line signifies the position of the overlapping number of DEGs that we found of 115.

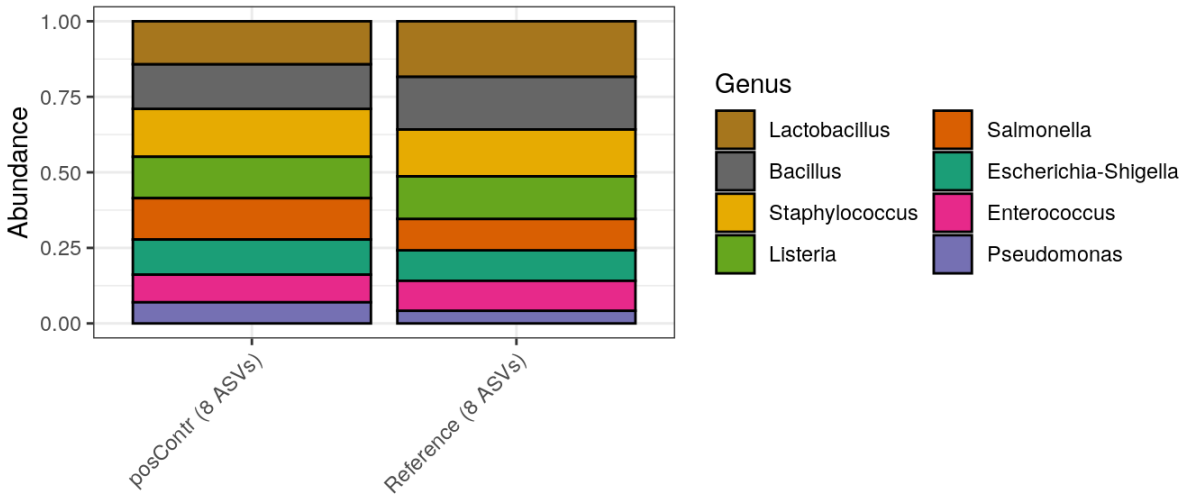

Figure S4 relative abundances of the positive control (left) plotted together with the expected relative abundance in the ZymoBIOMICS Microbial Community (right).

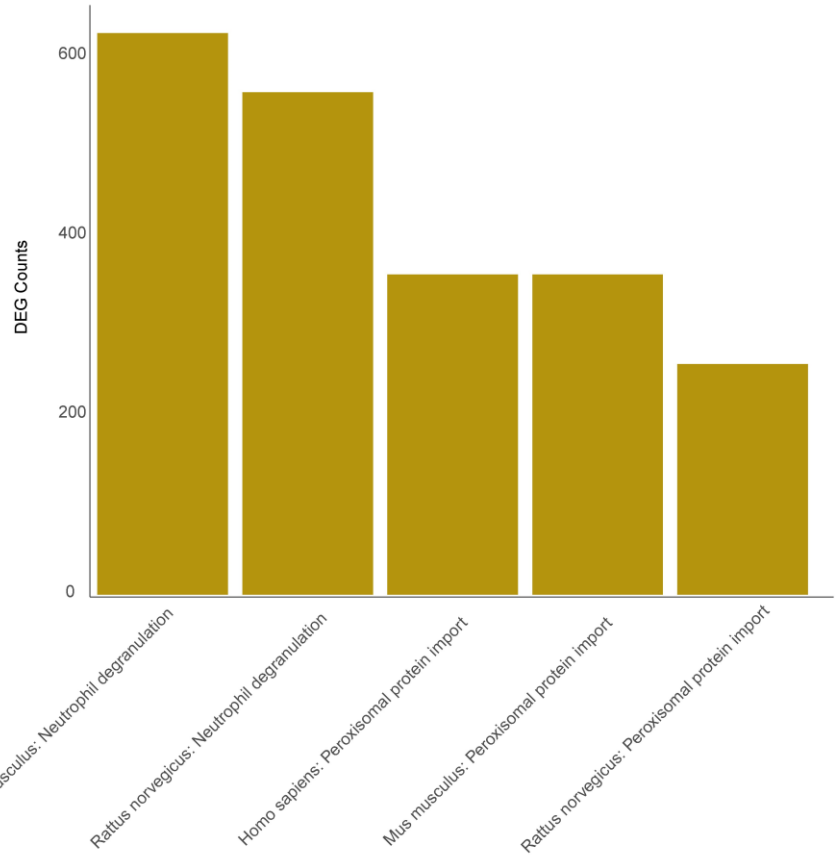

Figure S5 Bar plot representing the top 5 highest enriched Reactome terms for the cestode infection dataset.

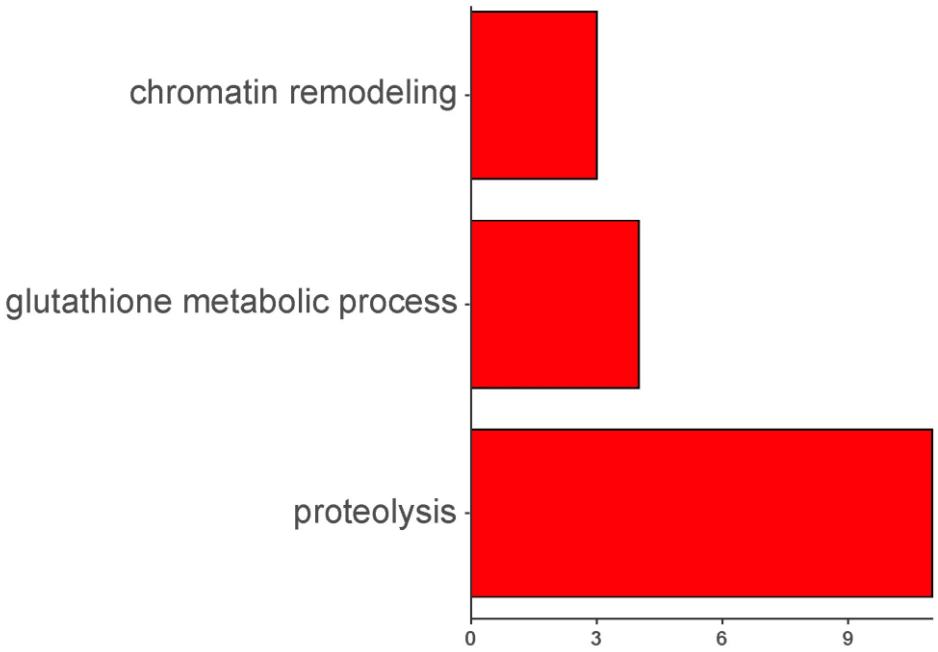

Figure S6 GO terms of the genes being co-differentially expressed in both cestode infection and LPS immune challenge where the X-axis represents the number of genes and the Y-axis represents the GO term.

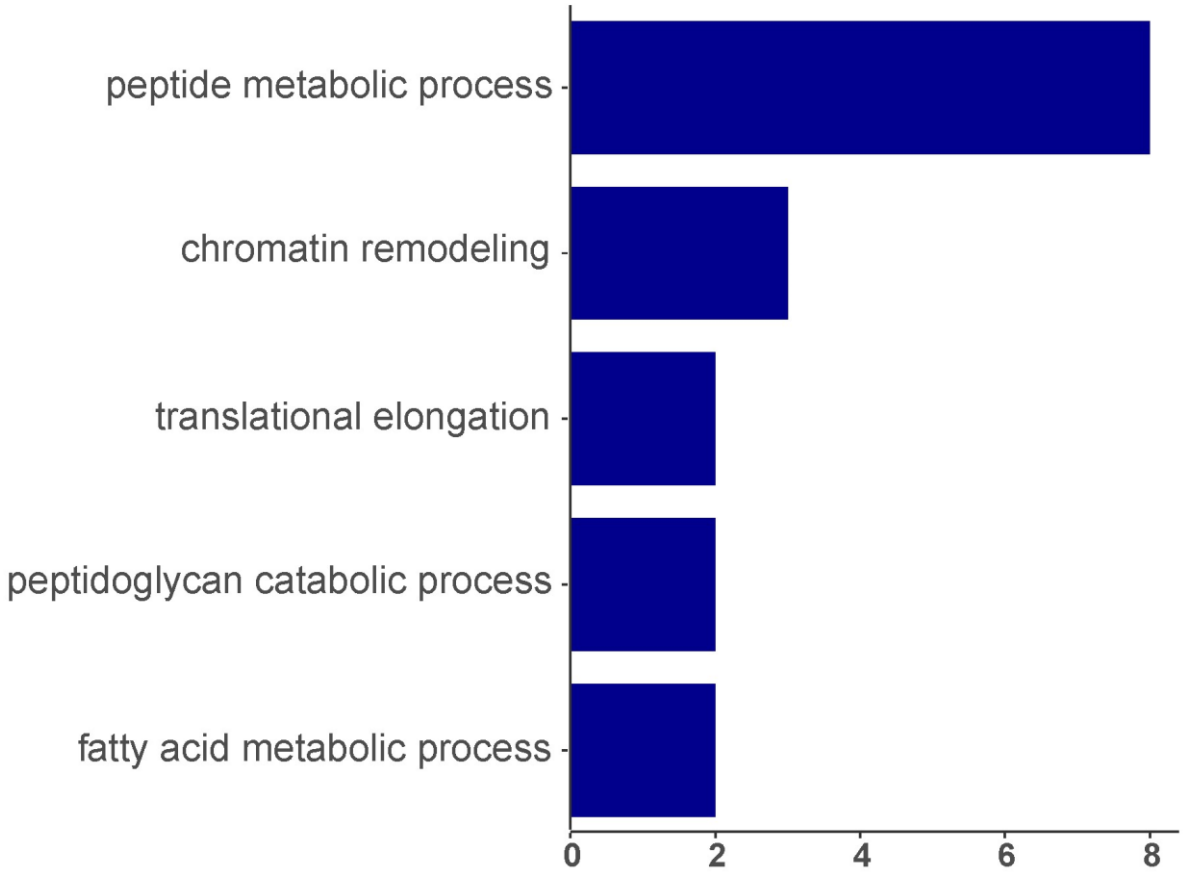

Figure S7 Significant GO terms for the LPS immune challenged and uninfected individuals with the GO terms on the Y axis and the number of representative genes on the x-axis. These GO terms only represent upregulated genes considering a lack of downregulated significant GO terms in this dataset.

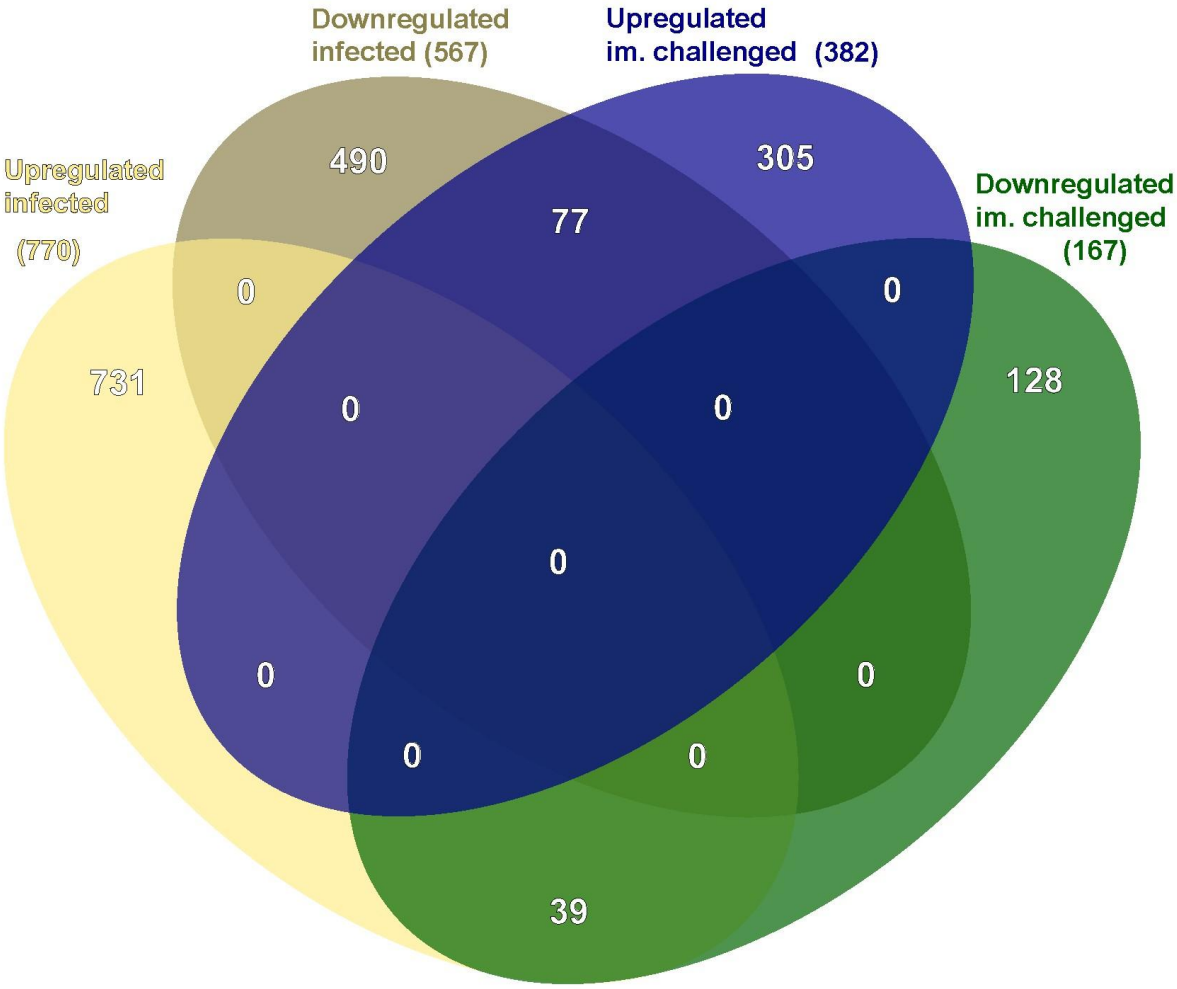

Figure S8 Venn diagram of the overlapping DEGs between LPS immune challenged and cestode infected comparisons. The overlap only being visible in opposite directions.

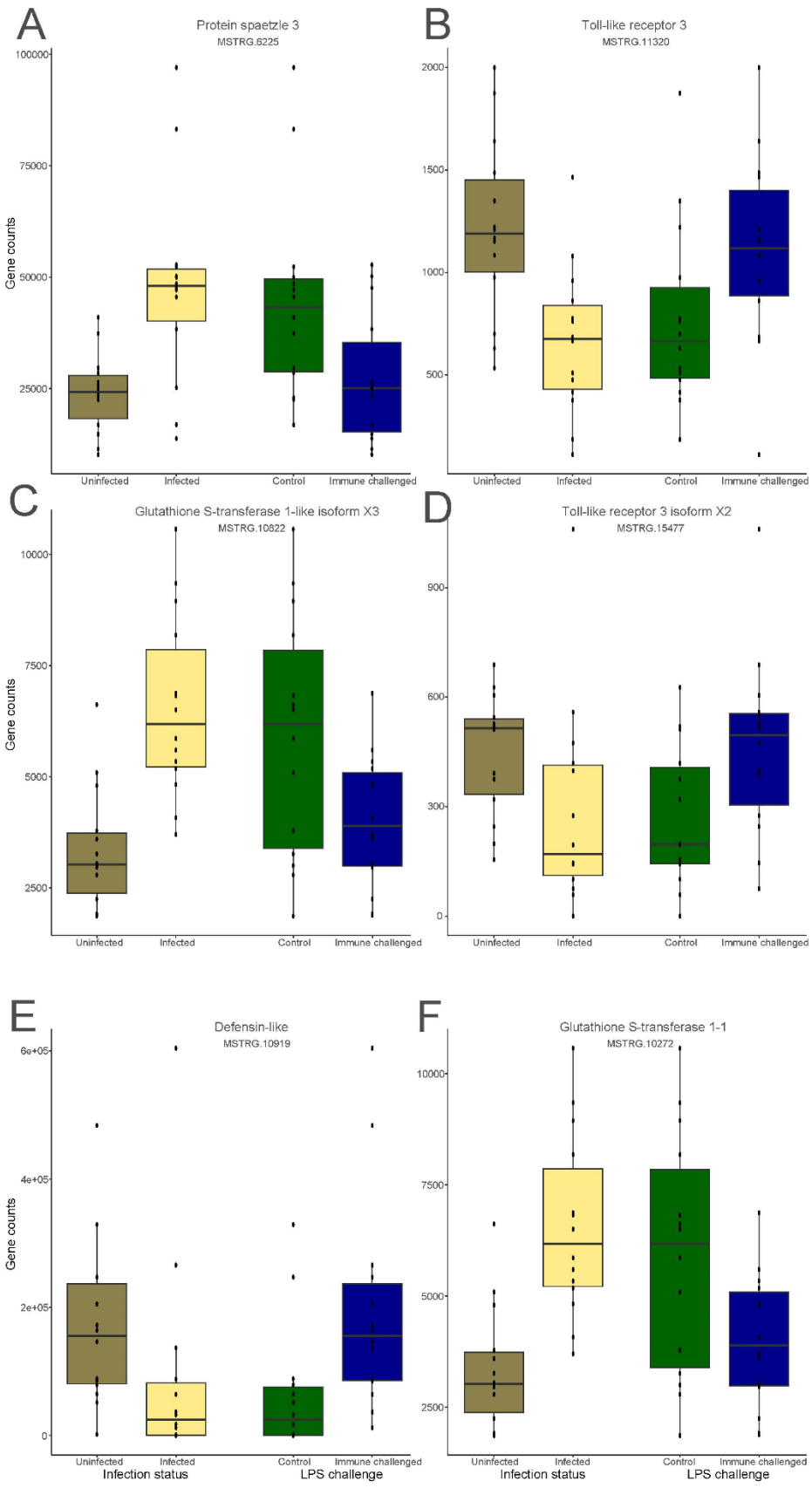

Figure S9 Six gene count plots for immune genes which were both significantly differentially expressed for cestode infection and LPS immune challenge. Thus the genes were differentially expressed in different directions. They are **A.** Protein spaetzle 3, **B.** Toll-like receptor 3, **C.** Glutathione S-transferase 1-like isoform X3, **D.** Toll-like receptor 3, **E.** Defensin-like and **F.** Glutathione S-transferase 1-1. The colours represent the groups as they have been in previous graphs.

Table S1 Information on the ant colonies gathered for this experiment.

Colony	Sampling date	No. of workers	No. of queens	No. of infected workers	Workers total	No. of infected Queens	Proportion infected	Notes	Cohort
A	9-6-2021	10	1	7	17		0.41		1
C	9-6-2021	21	1	9	30	3	0.3		1
E	8-6-2021	43	1	11	54		0.2		1
F	-	63	1	9	72		0.13		1
G	11-6-2021	97	1	21	118		0.18		1
L	9-6-2021	87	1	0	87		0		1
M	9-6-2021	52	1	0	52		0		1
N	11-6-2021	49	1	0	49		0		1
O	11-6-2021	22	1	0	22		0		1
P	11-6-2021	67	1	0	67		0		1
Q	11-6-2021	25	1	30	55		0.55	Has males	2
S	5-6-2021	30	1	0	30		0		2
T	-	107	1	12	119		0.1	Has males	2
U	5-6-2021	72	1	6	78		0.08	Has males	2
V	5-6-2021	64	1	0	64		0	Has males	2
X	5-6-2021	27	1	0	27		0		2

Table S2 Overview of individual samples used for the RNA seq and amplicon seq experiments. The samples marked with an asterisk are unique to the amplicon seq experiment.

	COLONY	INFECTED	TREATMENT	COLOUR OF MARKING
A1	LBW21imtnR1	Yes	LPS	Red
A2	LBW21imtnR1	Yes	none	Green
A3	LBW21imtnR1	No	LPS	Green
A4	LBW21imtnR1	No	none	Red
CO1*	na	Na	Dissection control	na
CO4*	na	Na	Dissection control	na
E1	LBW21ImTnR5	Yes	LPS	Red
E2	LBW21ImTnR5	Yes	None	Green
E3	LBW21ImTnR5	No	LPS	Green
E4	LBW21ImTnR5	No	None	Red
F1	LBW21ImTnR6	Yes	LPS	Red
F2	LBW21ImTnR6	Yes	None	Green
F3	LBW21ImTnR6	No	LPS	Green
F4	LBW21ImTnR6	No	None	Red
G1	LBW21ImTnR7	Yes	LPS	Red
G2	LBW21ImTnR7	Yes	None	Green
G3	LBW21ImTnR7	No	LPS	Green
G4	LBW21ImTnR7	No	None	Red
M1	LBW21ImTnR13	No	LPS	Red
M2	LBW21ImTnR13	No	None	Green
N1	LBW21ImTnR14	No	LPS	Green
N2	LBW21ImTnR14	No	None	Red
NC1*	na	Na	DNA isolation control	na
NC2*	na	Na	DNA isolation control	na
NEGB*	na	Na	Negative control	na
NEGC*	na	Na	Negative control	na
O1	LBW21ImTnR15	No	LPS	Red
O2	LBW21ImTnR15	No	None	Green
P1	LBW21ImTnR16	No	LPS	Green
P2	LBW21ImTnR16	No	None	Red
POSCONTR*	na	Na	Positive control	na
Q1	LBW21ImTnR17	Yes	LPS	Red
Q2	LBW21ImTnR17	Yes	None	Green
Q3	LBW21ImTnR17	No	LPS	Green
Q4	LBW21ImTnR17	No	None	Red
S1	LBW21ImTnR19	No	LPS	Red
S2	LBW21ImTnR19	No	None	Green
T1	LBW21ImTnR20	Yes	LPS	Green
T2	LBW21ImTnR20	Yes	None	Red
T3	LBW21ImTnR20	No	LPS	Red
T4	LBW21ImTnR20	No	None	Green
U1	LBW21ImTnR21	Yes	LPS	Green

U2	LBW21ImTnR21	Yes	None	Red
U3	LBW21ImTnR21	No	LPS	Red
U4	LBW21ImTnR21	No	None	Green
V1	LBW21ImTnR22	No	LPS	Green
V2	LBW21ImTnR22	No	None	Red
X1	LBW21ImTnR24	No	LPS	Red
X2	LBW21ImTnR24	No	None	Green

Chapter 3

Transcriptional networks indicate regulatory functions of parasite genes and the importance of infected worker prevalence for gene expression of host ants, uninfected nestmates and parasites

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In preparation

Author contributions

The study of parasite prevalence was conceptualized by **T.S.,** [redacted]. Sampling was done by **T.S.** [redacted]. The experiments were performed by **T.S.** The prevalence transcriptome analysis was done by **T.S.** with consultation of [redacted]. The cestode genome was generated by [redacted]. The interactome analysis was conceptualized and performed by **T.S.** with input of [redacted]. The draft was written and edited by **T.S.** with revision comments from [redacted].

Abstract

Parasites are not only a problem for the individual infected host, but - in the case of social species - also for the entire group. Not only human societies, but also social insects have evolved complex behavioural and physiological responses to parasite infections. This experimental study focussed on the consequences of a non-directly transmissible parasite, the cestode *Anomotaenia brevis*, on colonies of the ant *Temnothorax nylanderi*. This focus is particularly interesting in this system, as the well-cared-for infected workers live longer than their uninfected nestmates, who suffer from increased mortality. We tested whether the prevalence of infected workers, i.e. the ratio of infected to healthy colony members, affects gene expression of infected workers, their nestmates and the larval stage of the parasite itself. The infected worker prevalence indeed influenced the molecular physiology of infected ants and their healthy nestmates. With increasing prevalence, all ants, infected and not, showed signs of stress, indicating that the colony as a whole suffers from a high proportion of infected individuals. Moreover, the transcriptional activity of parasitic cestodes shifted with infected worker prevalence. A novel joint network analysis of parasite and host transcriptomes revealed that cestode genes act as hub genes that are closely linked to the activity of host genes. This points to regulatory functions of specific parasite genes and sheds new light on the molecular communication between parasites and hosts. The joint transcriptome analysis using weighted gene co-expression networks is proposed as a fruitful approach to investigate molecular interactions in host-parasite systems.

Key words: prevalence, interactome, parasite ecology, cestode, social insects, WGCNA

Introduction

No organism lives in its own universe, which is one of the most important principles of ecology. The disappearance or introduction of a species into an ecosystem often has profound consequences for sympatric species (Crowl et al. 2008; Zavaleta et al. 2009). This is particularly true for parasites, whose presence can influence the dynamics of host populations (Sinclair, 1979; Thomas & Bonsall, 2005), can alter competition between species (Price et al. 1986) and even shift ecosystem energy flows (Odum & Barrett, 1971). Parasites are thus important drivers of biodiversity and evolution (Hudson et al. 2006; Mouritsen & Poulin, 2002). In fact, one reason why invasive species are so successful in their new ecosystems is the absence of parasites and other antagonists as described by the enemy release hypothesis (Crawley, 1997; Keane & Crawley, 2002; Maron & Vilà, 2001). However, this also means that the influence of parasites not only affects their direct hosts, but also other organisms in their social groups and communities.

Social animals are often particularly affected by parasites and pathogens (Hart & Hart, 2018), as frequent close contact increases the transmission and spread of pathogens (Romano et al. 2022). The pathogen risk for these groups increases with increasing size and relatedness (Webber et al. 2017). Therefore, all social animals have developed not only physiological but also behavioural defence mechanisms, such as frequent grooming, self-medication, and changes in the frequency of interactions (Roode et al. 2013). This applies in particular to the influence of parasites on the behavior of eusocial insects. Insect societies are often referred to as superorganisms because their fitness is highly linked to colony performance (Hamilton, 1964; O'Shea-Wheller et al. 2021). The multifaceted behavioural defences against pathogens of social insects are often referred to as social immunity (Cremer et al. 2007; Cremer et al. 2018) and include changes in the interaction network structure (Stroeymeyt et al. 2014), which is comparable to quarantine in human societies, adapted grooming behaviour (Stock et al. 2023) or prophylactic distribution of antipathogenic substances (Chapuisat et al. 2007).

Many parasites are directly transmissible within a social group, and these require specific changes in the interaction behaviour to minimise their spread within the colony. Other pathogens are not transmissible, but can still affect the whole colony through their detrimental effect on infected animals. One example of this is the cestode *Anomotaenia brevis*, which uses the ant species *Temnothorax nylanderi* as an intermediate host and affects the behaviour, molecular physiology and life history traits not only of infected ants, but also of their healthy nestmates (Beros et al. 2015; 2021; Feldmeyer et al. 2016). 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). Nevertheless they show a several fold increased lifespan (Beros et al. 2021). This is possible as their uninfected nestmates take particularly good care of infected group members (Beros et al. 2021). The phenotypic traits of the uninfected workers also shift with colony infection, as they exhibit compared to healthy workers from uninfected colonies a lower aggression towards non-nestmates, alter their transcriptional activity in the brain and suffer from a shortened lifespan (Beros et al. 2021). Finally, infection of some workers with *A. brevis* has clear consequences on the colony level, as infected colonies produced sexuals with a male-biased sex ratio and are more likely to raise

intercaste individuals, i.e. females morphologically in-between workers and queens - both signs of a stressed colony (Scharf et al. 2012).

But how are these far-reaching consequences on the colony level possible? *T. nylanderi* ants become infected when they are fed with cestode eggs as larvae. The tapeworms then develop into cysticercoids which live in the ants' haemolymph until the final host, woodpeckers, preys on those infected workers. However, *A. brevis* cysticercoids do not remain dormant in their intermediate hosts but are physiologically active and secrete proteins into their host, so that 7% of all proteins in the haemolymph of infected workers originate from the tapeworm (Hartke et al. 2023). Thus not only changes induced during the development of infected workers, but also those due to active secretion in adult hosts lead to transcriptional changes in the brain, gaster and fat body of infected host workers (Feldmeyer et al. 2016; Sistermans et al. 2023; Stoldt et al. 2021). In particular, recent analyses show that parasite infection causes a suppression of the immune system (Sistermans et al. in prep). This system is also dynamic, as parasite load can vary between a single cestode infecting an individual ant and up to 74 cysticercoids (Sistermans et al. 2023). As in respect to other phenotypic traits, these transcriptional shifts do not only occur in infected workers, but also in uninfected ants from infected colonies (Sistermans et al. 2023). Recent re-analysis of survival data (Beros et al. 2021) also indicate that a high prevalence of infected workers in infected colonies negatively influence the survival of healthy nestmate workers and queens (Rognet & Kokko pers. inform.). And there are large differences in prevalence across colonies: In some colonies there is only a single infected worker, while in others up to 70 % of the workers are infected.

Variations in the prevalence of infected workers at the colony level create an additional dimension in this parasite-host system that could provide new insights into the ability of *A. brevis* to manipulate its intermediate host. A high prevalence of infected ants in a colony should lead to higher costs for uninfected nestmates, who now have to spend more time and resources caring for their infected nestmates, resulting in higher mortality rate (Rognet & Kokko, pers. inform.). In infected ants, this could lead to increased competition for care and food. Finally, one might expect that parasites in colonies with a higher prevalence should have a stronger influence on certain traits of their host. Many of the changes in host phenotype, such as increased longevity (Beros et al. 2021), reduced escape behaviour (Beros et al. 2015) and changes in immunity (Sistermans et al. in prep)., suggest that the parasite is the one pulling the strings behind these changes. Therefore, we aim to identify the consequence of parasite prevalence at colony level on the transcriptional activity of infected and uninfected workers and the parasite itself. Since the ability of uninfected nestmates to care for infected nestmates should depend on the size of the colony, we decided to include colony size and its interaction with the infected worker prevalence in our transcriptional analyses. The interaction here means that we test whether a high prevalence has stronger effects in smaller or larger colonies. It is conceivable that larger colonies are better able to buffer the stress caused by the presence of many infected animals.

This study also provides us with a special opportunity to investigate the interaction between parasite and host at the transcriptomic level, as we have analysed the gene expression in the fat body of host workers and their parasitic cysticercoids, respectively. How the molecular physiology of hosts and their

parasites are interlinked has been difficult to answer. Some studies used protein-protein interaction predictions (Davis et al. 2009; Dyer et al. 2007; Soyemi et al. 2018), a method which is prone to a high false discovery rate (Lin et al. 2004; Lu et al. 2005). Instead, we will perform a joint network analysis of transcriptional data of cestodes and ants from the very same individuals. In this way, we can identify candidate parasite genes whose expression is linked to many host genes to gain insight into whether or how *A. brevis* actually pulls the strings or whether these induced changes are side effects of parasite infection.

Material and methods

Sampling, experimental design and sequencing

We collected *Temnothorax 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). In the laboratory, the ant colonies were transferred to artificial nest sites consisting of a Plexiglas perimeter and a lid and base made of a microscope slide. These nest sites were covered with a red foil and transferred to a three-chambered nest box with a plaster floor. Each ant colony was fed twice weekly with crickets and honey and provided with water ad-libitum. We counted the number of queens, healthy and infected workers and selected 15 colonies whose infected worker prevalence varied between 2 % and 72 %. These 15 focal colonies also varied in colony size with between 16 and 177 workers (median number of workers 77). Infected workers can be recognized undoubtedly by their yellow cuticle (Scharf et al. 2012). A low single-digit percentage of workers with a healthy phenotype may also be infected with the cestode *A. brevis* in infected colonies (Scharf et al. 2012). As these can only be identified by mass dissection, we relied on the external phenotype to calculate the prevalence of infected workers. Yet, for our transcriptome analyses we ensured by dissection that healthy workers were indeed uninfected. All infected workers and a comparable number of uninfected workers were dissected at random in the summer of 2020. We isolated the fat body from each worker by removing the gut and trachea from the first gaster segment, leaving only the fat cells, and placed those in a vial containing 50 µl Trizol. In infected ants, we also removed the cestodes, which are located in the haemolymph around the midgut and placed them in another vial with 50 µl Trizol. Samples were frozen on dry ice and transferred to -80°C. We selected one infected worker per colony, whose cestode number was closest to the median of 7 (min=5, max=13). For each colony, we also included all cestodes of this worker and the fat body of an uninfected nestmate. We extracted RNA using the Qiagene RNeasy extraction kit according to protocol specifications and sent it to Novogene (Cambridge, UK) for sequencing. RNAseq data for four of the uninfected workers were also used in Sistermans et al. (2023), which were obtained using the same protocol (Table S1 for a detailed overview). For the low amounts of cestode RNA, a SMARTer amplification step was first performed. Thereafter, all samples underwent the same treatment: mRNA was enriched with oligo(dT) beads and randomly fragmented by adding fragmentation buffer. Subsequently, cDNA was synthesized using an mRNA template and a random hexamer primer. A customized second-strand synthesis buffer (Illumina), dNTPs, RNase H and DNA polymerase I were added to initiate second-strand synthesis. To complete the preparation of cDNA libraries, terminal repair, A-base and sequencing adapter ligation,

size selection and PCR enrichment were performed. The libraries were sequenced on an Illumina Novaseq 6000. We obtained at least 9 Gb per sample, resulting in approximately 30 million paired-end 150 bp reads.

Cestode genome assembly and mapping of RNA reads

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 ultra-low 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). After genome annotation we mapped the reads using STAR v 2.7.10b (Dobin et al. 2013). For the genome fasta file, we used the cestode genome assembly and a *T. nylanderi* genome assembly (Jongepier et al. 2022) in order to filter out reads that mapped either to both assemblies or to the ant genome. We constructed a gene count matrix using htseq (Zanini et al. 2022) and created a CDS file using gffread and the genome annotation and assembly (Pertea & Pertea, 2023), which we filtered for the largest isoform per gene. We used this file to BLAST the genome using BLAST diamond (Buchfink et al. 2014). We performed functional annotation on the CDS file for both GO and KEGG IDs using Eggnog mapper (Cantalapiedra et al. 2021).

For the ant reads, we first removed sequences from *A. brevis, Homo sapiens, Escherichia coli,* adaptors and vectors from the raw reads using fastqscreen v0.14.0 (Wingett & Andrews, 2018). The remaining sequences were trimmed using fastp (Chen et al. 2018) for 120 bp or more with an N-base cut-off of 15. After trimming we assessed read quality using fastqc (Andrews, 2010) to identify potential outliers, which we did not. We then mapped the reads against a genome assembly of *T. nylanderi* (Jongepier et al. 2022) with hisat2 v2.1.0 (Kim et al. 2015) using the —dta parameter. The hisat output was sorted with samtools (Li et al. 2009). The sorted BAM files were used to generate a genome-guided transcriptome assembly with stringtie v1.3.6 (Pertea et al. 2015). Transcript sequences were extracted using gffread v0.11.4 (Pertea & Pertea, 2023). For the construction of a gene count matrix we used the prepDE.py script from stringtie (Pertea et al. 2015). We constructed a filtered transcriptome with seqtk (Li, 2013) using the transcript file from stringtie, which we blasted with BLAST diamond (Buchfink et al. 2014). We performed functional annotation using eggnog mapper (Cantalapiedra et al. 2021) for KEGG pathways and GO terms.

Differential gene expression analysis

We split the ant data set into infected and uninfected samples, as the difference between infected and infected workers has been analysed at length elsewhere (Sistermans et al. 2023). We created PCAs for

the infected and infected 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 (Love et al. 2014). We performed a likelihood ratio test (LRT) linking gene expression to log-transformed infected worker prevalence using parasite load (number of cysticercoids per ant) and colony size (total number of workers) as reduced factors. Benjamini-Hochberg-adjusted p-values were extracted to identify differentially expressed genes (DEGs). We chose log-transformed values of infected worker prevalence in order to obtain a more even distribution of the prevalence data so that individual outliers do not lead to distortions. The distribution after log-transformation was closer to a normal distribution (Fig. S1). We also tested the effect of colony size by taking log-transformed prevalence and parasite load as reduced factors. The influence of an interaction between log prevalence and colony size was tested using log prevalence, parasite load and colony ID as reduced factors. Here we also checked for overlapping DEGs in the three datasets making a venn-diagram using InteractiVenn (Heberle et al. 2015). To assess in which way transcriptome shifts in response to infected worker prevalence were modified by colony size, we partitioned data set 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. Due to the accounting of GO topology we decided to not apply multiple testing correction on the GO terms because the p-values gained from this method are not independent. We considered only p-values below 0.01 in the GO enrichment analysis. We conducted a KEGG pathway enrichment analysis using clusterProfiler (Wu et al. 2021), where we corrected for multiple testing using the Benjamini-Hochberg method. We then applied the same analytical procedures for the cestode, and uninfected ant reads, with one exception. For the data set of uninfected workers, some of which were sequenced in 2020 and 2022, we also included sequencing year as a reduced factor in all models and checked its influence using PCA (Fig. S2).

We compared gene expression patterns between infected and uninfected workers as a function of the two main factors and their interactions a well as the overlap of differentially expressed genes. However please note, that the models for both data sets are not completely identical, as the number of cestodes was also included in the model for the infected workers and the year of sequencing for the uninfected workers. This may affect the analytical performance of the models and the results. Therefore, we only report on the overlap but do not test whether it is larger than expected as we used different models and therefore the expectations are unclear.

Joint parasite-host-coexpression analysis

We used the co-expression network analyses (WGCNA; Langfelder & Horvath, 2008) to identify candidate genes involved in communication between *A. brevis* and *T. nylanderi* and possibly in host manipulation. First, we combined the gene count matrices of cestodes and ants, per sample, i.e. for the fat body sample A6 we also used the cestode sample A6, as they both originate from the same

ant. This resulted in a gene count matrix for our 15 infected joint samples which contained both cestode and ant genes. From this joint gene count matrix, we then filtered out genes with less than 10 counts for at least 5 samples and checked the data for missing entries with a verbosity value of 3. To construct an unsigned co-expression network, we set the soft-thresholding power to 8 and, after testing multiple module sizes, set the minimum module size to 100 based on confirmation by a TOM plot. We merged modules with a dissimilarity threshold of 0.2 and verified this with another TOM plot (Fig. S2). We identified hub genes by selecting genes with 10 % highest connectivity. We used a chi-square test per module to check whether there were more cestode hub genes than would be expected by chance. For this, we used the proportion of cestode genes per module as the expected variable, the proportion of cestode hub genes as the observed variable and the number of hub genes as the sample size. We corrected the p-values for multiple testing using Benjamini-Hochberg adjustment. We then converted our topological overlap matrix into Cytoscape objects (Shannon et al. 2003) for each module to identify correlations between two genes and their weight. In this way, we were able to visually confirm a deeper integration of specific cestode genes into ant gene networks and vice versa. The cytoscape objects were also used to test whether genes from one species within a module are more likely to interact with genes from the other species. For this, we assume that each gene should interact with a random selection of other genes in the module. So, if we have a hypothetical module with a ratio of 50:50 ant : cestode genes, the null hypothesis would be that a gene of whatever origin should interact half and half with cestode and host genes. Perhaps we could even hypothesise that there should be more interactions between genes of the same species, but this was difficult to implement. We therefore determined the proportion of genes interacting with cestode genes for all ant and cestode genes in each module and tested whether their proportions differed from the assumptions of the null hypothesis for each species using the chi-square test with the number of ant or cestode genes in each module as the sample size. We then corrected the p-values for multiple testing using the Benjamini-Hochberg method.

In addition, we investigated genes of the cestode that are actively released as proteins into the host haemolymph (Hartke et al. 2023). We selected the 15 most abundant cestode genes in the ant haemolymph proteome and all annotated genes among the 50 most abundant genes. We used these genes as queries for a diamond BLAST database that we created using our genome cds file, which we first translated using TransDecoder v5.5.0 (Haas et al. 2013). From the BLAST hits, we selected the most frequently expressed gene for each hit to identify the genes in the proteome. Using these genes, we first checked which module they were in and assessed whether these modules contained ant or cestode gene clusters that were more strongly associated with the other species. We also calculated a confidence interval for these modules and tested whether these genes were more likely to interact with the other species within the clusters by confirming that these genes fell outside the 95% confidence interval. We also identified the ant genes these cestode genes interacted with and performed GO and KEGG enrichment analyses for these ant genes to assess their potential functions.

Results

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

After splitting the dataset, we visualized the data distribution of the infected worker samples in response to prevalence and colony size (Fig. 1a). We used DESeq2 to identify genes whose expression in infected workers was linked to the prevalence of infected workers in the colony. This analysis resulted in a large number of differentially expressed genes, namely 647 DEGs, of which 339 were upregulated and 308 were downregulated. The KEGG pathway analysis identified no enriched KEGG terms after p-adjustment. In fact, no significant KEGG paths were found in any of our data sets, which we will therefore not discuss further. However, we identified 19 significantly enriched GO terms (Fig. 1e), eleven for the upregulated and eight for the downregulated genes. Several of these GO terms are related to peptide and protein transport, including *peptide transport* with nineteen upregulated genes, *targeting of proteins to the ER* with five upregulated genes, and *processing of signalling peptides* with three upregulated genes. Additionally, we uncovered functions related to lipogenesis like *lipoxygenase pathway* and *lipoxin biosynthetic process* with each two upregulated genes and *negative regulation of fatty acid transport* with two downregulated genes. Shifts in fatty acid transport genes may indicate that infected workers alter their need for fat burning with prevalence, as this term is associated with the transport of fatty acids to the mitochondria (Marshall et al. 2014). The fat body tissue plays an important role in insect immunity (Hoffmann & Reichhart, 2002). Indeed, lipoxygenase products are important for eicosanoid signalling, involving both the lipoxygenase pathway and the lipoxin biosynthesis process (Kim & Stanley, 2021). In addition, we found other immune processes also to be involved, such as *IRES-dependent viral translation initiation* with two upregulated genes and *positive regulation of NIK/NF-kappaB signalling* with three downregulated genes. The latter signalling process is linked to innate and adaptive immune responses as a mediator of inflammatory responses (Liu et al. 2017). In order to gain a better understanding on whether starvation might be responsible for this fat burning reaction and find out what direction this starvation response might go we decided to plot several genes that are differentially regulated and investigate their BLAST functions (Fig. S5). The first of these genes is CCAAT/enhancer-binding protein (Fig. S5a) whose upregulation is capable of protecting cells against energy starvation (G. Lu et al. 2014). This gene is also involved with the protection against toxic substances from microbial pathogens (Reddy et al. 2016). The second gene, Zinc finger protein 662-like, is a protein that promotes stress-induced mitochondrial longevity (Merkwirth et al. 2017). This could be among others starvation stress and zinc finger nuclease cleavage of Bak and Bax proteins makes cells more resistant to starvation-induced apoptosis (Cost et al. 2010), in our dataset this gene was downregulated but the inhibition of this gene in starvation has not been investigated. Finally, we plotted flavin reductase (Fig. S5c), which was also significantly downregulated, the overexpression of flavin reductase increases the stress tolerance including starvation stress (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 (Fig. 1c). One of them was annotated as a *facilitated trehalose transporter Tret1-2*

homologue, which appears to be involved in cellular protection against desiccation (Kikawada et al. 2007).

In the transcriptomes of infected ants we identified 287 genes, whose expression was influenced by an interaction between colony size and prevalence. GO enrichment analyses uncovered no terms with more than one gene to be enriched with a Fisher's p-value below 0.01. When we analysed 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, suggesting that larger colony size might buffer the expression changes of this gene with infected worker prevalence. This gene did not result in an annotated BLAST result.

The principal component analysis of uninfected worker samples, revealed two outliers (Fig. 1b). As neither entries in our lab books nor quality control did explain their divergent gene expression, we found no external reason to remove them from our data set and they remained in all subsequent analyses. The transcriptomes of uninfected workers were also influenced by the prevalence of infected workers in the colony. Here, 133 genes significantly changed their expression only as a function of prevalence, of which 52 were downregulated and 79 were upregulated. We found eight GO terms in the upregulated group with increasing prevalence, all of which were represented by two genes (Fig. 1f). At least three of these terms indicate stress, e.g. *response to aluminium ions*, which is usually induced during aluminium stress causing an accumulation of reactive oxygen species (ROS) 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). And 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 be 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 (Fig. 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 (Fig. 1g). With colony size, many genes changed their expression with neuronal functions. These terms include *neuron formation* (48 upregulated genes), *brain development* (21 upregulated genes), *adult locomotor behaviour* (11 upregulated genes), *neurotransmitter secretion* (eight upregulated genes), *neuromuscular synaptic transmission* (8 upregulated genes), *regulation of synaptic plasticity* (seven upregulated genes), *behavioural response to ethanol* (5 upregulated genes), several other synaptic functions with five upregulated genes and *flight behaviour* (5 upregulated genes). These neurological functions may be prevalent due to the fact that 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). It is therefore not surprising that we also uncover the *positive regulation of insulin secretion* (five upregulated genes) for these DEGs. Other

terms include *antifungal humoral response* with three 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 (Fig. S4a) revealed that a number of these related to the defence 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 signalling pathway* (2 genes). Two of the DEGs we found to be differentially expressed in the small colonies out of seventeen genes in total and one of which in the large colonies out of eight genes in total. 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 signalling 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, 0 for colony size and 8 for the interaction. As noted in the methods, this comparison is somewhat problematic as the two models are not identical but include parasite load in one and sequencing year as reduced factors in the other.

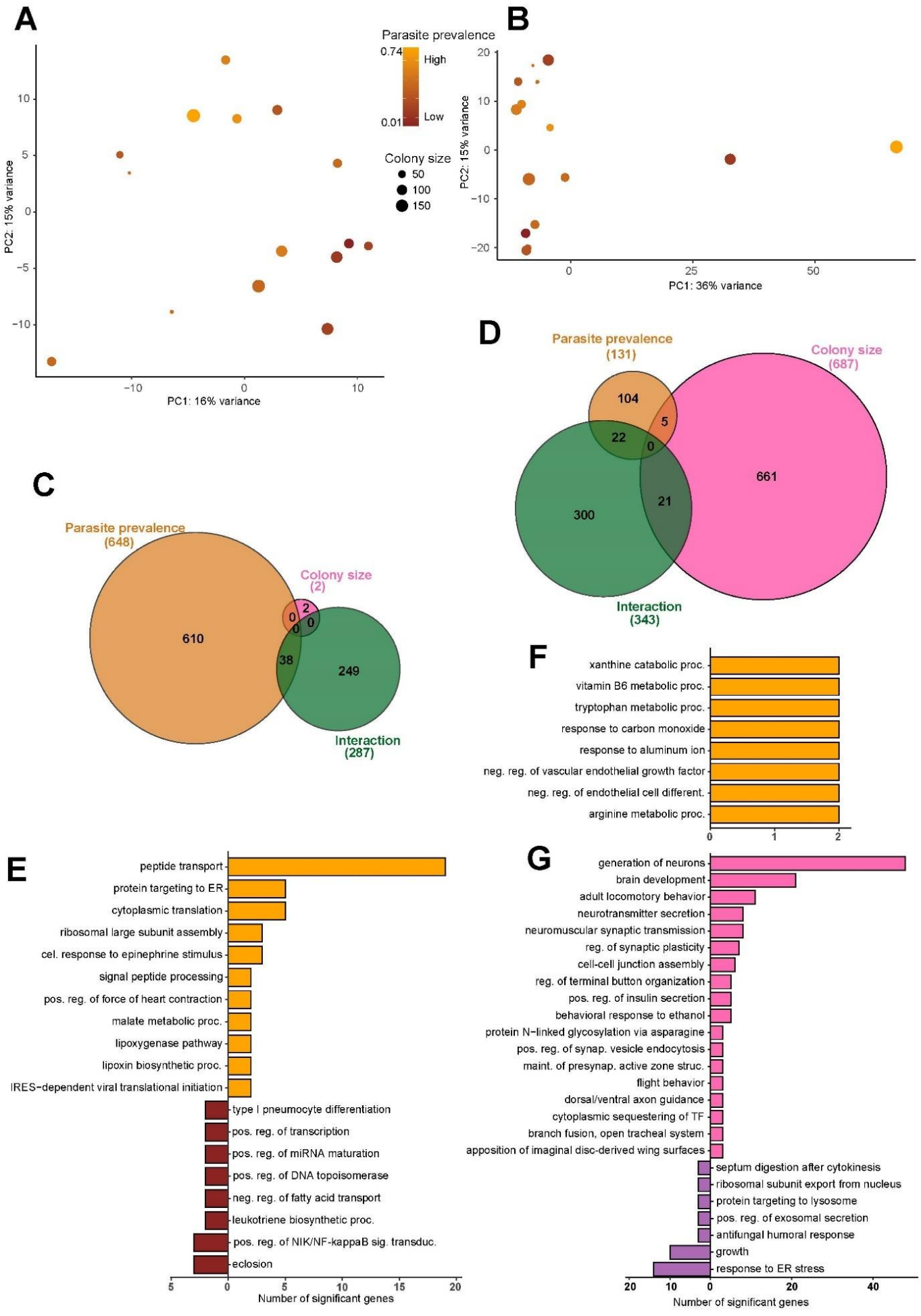
A
Parasite prevalence
0.74
High
0.01
Low
Colony size
50
100
150
PC2: 15% variance
PC1: 16% variance
B
PC2: 15% variance
PC1: 36% variance
C
Parasite prevalence
(648)
Colony size
(2)
610
2
0
0
0
38
249
Interaction
(287)
D
Parasite prevalence
(131)
Colony size
(687)
104
5
22
0
661
21
300
Interaction
(343)
E
peptide transport
protein targeting to ER
cytoplasmic translation
ribosomal large subunit assembly
cel. response to epinephrine stimulus
signal peptide processing
pos. reg. of force of heart contraction
malate metabolic proc.
lipoxygenase pathway
lipoxin biosynthetic proc.
IRES−dependent viral translational initiation
type I pneumocyte differentiation
pos. reg. of transcription
pos. reg. of miRNA maturation
pos. reg. of DNA topoisomerase
neg. reg. of fatty acid transport
leukotriene biosynthetic proc.
pos. reg. of NIK/NF-kappaB sig. transduc.
eclosion
Number of significant genes
F
xanthine catabolic proc.
vitamin B6 metabolic proc.
tryptophan metabolic proc.
response to carbon monoxide
response to aluminum ion
neg. reg. of vascular endothelial growth factor
neg. reg. of endothelial cell different.
arginine metabolic proc.
G
generation of neurons
brain development
adult locomotory behavior
neurotransmitter secretion
neuromuscular synaptic transmission
reg. of synaptic plasticity
cell-cell junction assembly
reg. of terminal button organization
pos. reg. of insulin secretion
behavioral response to ethanol
protein N−linked glycosylation via asparagine
pos. reg. of synap. vesicle endocytosis
maint. of presynap. active zone struc.
flight behavior
dorsal/ventral axon guidance
cytoplasmic sequestering of TF
branch fusion, open tracheal system
apposition of imaginal disc-derived wing surfaces
septum digestion after cytokinesis
ribosomal subunit export from nucleus
protein targeting to lysosome
pos. reg. of exosomal secretion
antifungal humoral response
growth
response to ER stress
Number of significant genes

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, colour signifies parasite prevalence with lighter colours representing more heavily infected colonies, dot size represents colony size with larger dots being from larger colonies. **B.** Principal component analysis of the uninfected ant transcriptome. **C** and **D.** Venn diagrams showing the differentially expressed genes depending on prevalence of infected workers, colony size and their interaction in **C.** infected workers and **D.** uninfected workers **E.** GO terms for the infected ants for the prevalence analysis, the orange bars being the gene counts of upregulated DEGs and the brown bars being the gene counts of downregulated DEGs. **F.** GO terms for the uninfected ants for the parasite rate analysis where we only found enrichments for the upregulated genes with parasite rate. **G.** GO terms for the uninfected ants in the colony size analysis where the hot pink bars represent the genes upregulated with colony size and the purple bars represent the genes downregulated with colony size.

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 in the colony and colony size (Fig. 2a). When analysing the effects of prevalence on the cestodes, we found 64 DEGs, of which 18 were upregulated and 46 were downregulated. Among the GO terms, we found only three in the downregulated genes (Fig. 2c), namely *positive regulation of cell motility* (3 genes), *sensory perception of pain* (3 genes) and *positive regulation of dendrite extension* (2 genes). Except for the regulation of cell motility, which is a subset of locomotion, the terms are associated with the nervous system, which seems surprising for cysticercoid larvae of a tapeworm. There are cysticercoid cestodes that target the hosts brain (Sukhdeo, 1992), but in our model system the parasites reside in the host gaster and thus an influence does not seem likely. What is more likely is that these neurological functions are related to the cestode's interaction to the enteric nervous system. Multiple parasites associated with the host gut are known to alter gastrointestinal motility, absorption and secretion through interaction with the enteric nervous system (Halliez & Buret, 2015).

Regarding the effect of colony size on cestode gene expression, we found a total of 116 DEGs, 74 of these genes were downregulated and 46 were upregulated, we only found three genes overlapping with parasite prevalence (Fig. 3b). Among the downregulated genes, we encountered a single enriched GO term, namely *lipid phosphorylation* with two representative genes. Phospholipids are important sources of energy for developing platyhelminths (Ghosh & Misra, 2014; Humiczewska & Rajski, 2005), and therefore the differential lipid phosphorylation is likely the 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 (Fig. S4b). These terms were sensory perception of pain and positive regulation of neuron apoptotic process, both again neurological functions. Separate analysis in small and large colonies revealed the influence of prevalence in particular for one DEG in small colonies and fifteen DEGs in large colonies, however, as can be seen in figure 2b, there was no overlap between the DEGs found in the interaction.

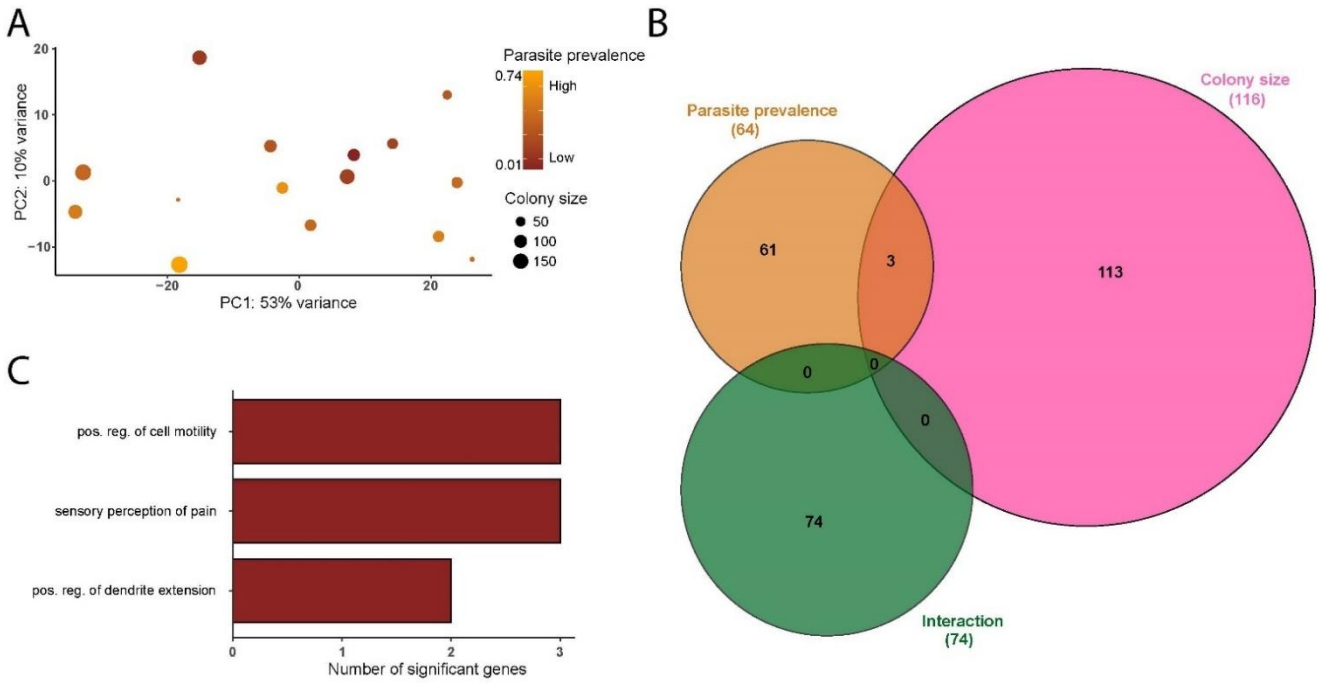

Figure 2 Cestode gene expression in relation to prevalence of infected workers in the colony. A. Principal component analysis of the cestode transcriptomes where colour signifies prevalence. Lighter colours are from cestodes from colonies with a higher prevalence of infected workers. Dot size signifies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

Parasite-host-co-expression analysis

We identified 18 WGCNA modules from a total of 20806 genes, of which 9472 were cestode genes. The module sizes ranged from 261 genes (grey) to 5751 genes (brown), (see Fig. 3a). These modules contained all genes of both species, with the proportion of cestode genes ranging from 0.02 (grey60) to 0.8 (turquoise). When identifying hub genes - genes with the 10% highest interactions - for all modules, we discovered that in most modules the proportion of hub genes differed significantly from that of all genes of the respective species in the module (Fig. 3b). In two modules, all hub genes were from the host, but in six modules all hubs were from cestode genes. In fact, 11 out of 18 modules had a significantly higher proportion of cestode hub genes, while the proportion of cestode genes in these 11 modules varied between 0.55 (brown module) and 0.8 (turquoise module).

When visualising the networks, we also discovered a high integration of interspecies gene correlations (Fig. 3c). This was also evident in the interaction analysis, where we tested whether genes of one species were more likely to interact with genes of the other species. Here, after p-adjustment, we identified six modules, in which ant genes were more likely to interact with cestode genes than expected at random (table 1). In contrast to the cestode clusters, where we did not find a higher-than-expected interaction with ant genes.

Table 1 General statistics about the interactome modules, in the two rows with the P-adjusted values for differential interaction the black values signify interactions more strongly towards the other species while the grey values signify stronger interactions towards the own species. Whenever a p-value is in bold it is either significant or the genes interact significantly more with the other species.

Module ID	Proportion of cestode genes	P-adjusted ant genes interacting differently than proportion	P-adjusted cestode genes interacting differently than proportion	P-adjusted cestode genes hub representation compared to proportion
Black	0.61	**2.47E-09**	1.15E-04	**9.41E-14**
Blue	0.06	9.8E-04	1	**2.10E-03**
Brown	0.55	**3.02E-67**	1.59E-18	**3.41E-72**
Cyan	0.79	**0.01**	1.10E-08	**7.34E-04**
Darkgreen	0.08	0.12	1	1
Darkorange	0.75	**1.76E-04**	3.44E-10	**4.85E-05**
Darkred	0.15	0.19	1	0.40
Greenyellow	0.79	0.07	1.36E-03	**3.06E-03**
Grey	0.23	0.31	1	1
Grey60	0.02	0.31	1	1
Lightcyan	0.54	**1.76E-04**	3.68E-04	**1.35E-06**
Lightgreen	0.19	3.04E-04	1	0.26
Lightyellow	0.75	0.11	1.02E-03	**0.03**
Magenta	0.09	7.72E-04	1	0.19
Midnightblue	0.03	0.31	1	1
Pink	0.08	2.11E-10	1	**0.01**
Royalblue	0.70	0.31	7.96E-04	**0.02**
Turquoise	0.80	**1.09E-10**	8.93E-08	**5.89E-13**

Interaction partners of abundant cestode genes in the ant haemolymph

A. brevis secrets proteins into the haemolymph of its intermediate host (Hartke et al. 2023). We identified four annotated and 12 unannotated transcripts of these proteins in our dataset (Fig. 3d) and we discuss the interaction partners for these genes separately. The four annotated genes are *thioredoxin peroxidase* and *superoxide dismutase* (black module), *lysosomal alpha-glucosidase* (brown module) and *putative protein disulfide isomerase ER 60* (dark orange module). These genes interacted

differently with other genes than their proportion in the modules would suggest, with *superoxide dismutase* interacting more with ant genes, while the others interacted more with cestode genes than expected. Two of these genes, *disulfide isomerase* and *alpha-glucosidase*, were also found to be hub genes. GO enrichments of the ant interaction partners of these genes (Fig. 3e) identified significant GO terms of their interaction partners.

We found for ant interaction partners of *superoxide dismutase* two terms, namely *cation transmembrane transport* (10 genes) and *response to electrical stimulus* involved in regulation of muscle adaptation (2 genes). *Superoxide dismutase* is known to regulate oxidative stress in

Caenorhabditis elegans (Yang et al. 2007), but its expression was also linked to a long lifespan in queens of several social insect species (Negroni et al. 2019; Parker et al. 2004; Tasaki et al. 2018). In parasitic helminths, it can play a role in defence against reactive oxygen species (ROS) originating from the host (Calvani et al. 2022). Transmembrane transport of cations, meanwhile, can cause an increase in ROS (Dow, 2017), and muscle contractions do the same (Powers et al. 2011), possibly explaining the link between this cestode gene and ant genes with those functionalities. In the same module (black), we found another antioxidant, *thioredoxin peroxidase* (McGonigle et al. 1997), which is involved in the regulation of oxidative stress associated with normal aerobic metabolism (Salinas et al. 1998). This corresponds to the enriched GO terms of the interacting host genes, which encompass several metabolic processes, such as *proteasome-mediated ubiquitin-dependent protein catabolism*, *peptidyl-threonine dephosphorylation* and *regulation of transcriptional start site selection at the RNA polymerase II promoter*. In addition to these processes, we find pathways in the ant itself that are involved in the defence against oxidative stress, such as ADP transport, in which ADP protects cells from oxidative stress (Sokolova et al. 2013). The agreement of these results with our findings for *superoxide dismutase* could also be due to the fact that many of the interacting host genes are the same. In fact, 190 out of 191 interacting partners of *superoxide dismutase* were also among the 428 interacting partners of *thioredoxin peroxidase*. The third gene, *putative protein disulfide isomerase ER 60*, belongs to a family of genes that affect parasite virulence (Achour et al. 2002) and its downregulation in parasitic nematodes can increase parasite mortality in host plants (Habash et al. 2017). The most common GO term, with four representatives, is *mating*, in which disulfide isomerases do appear to play a direct role. In vertebrates, disulfide isomerases were shown to be chaperones for ADAM3, the misfolding of which can lead to male infertility (Tokuhiro et al. 2012). In parasites, this gene can reduce host fertility by increasing the production of secretory proteins, which leads to a reduction in mating factor binding (Inan et al. 2006). This function as a chaperone for secretory proteins could explain the GO term *positive regulation of calcium ion-dependent exocytosis* with two genes. Exocytosis is an important part of the secretory pathway (Baker & Knight, 1986).

The last annotated gene for which we identified interaction partners is *lysosomal alpha-glucosidase* from the brown module, which is a basal metabolism gene that breaks down glycogen and releases glucose from long-term energy storage (Hermans et al. 1991). In cestodes, the expression of another glucosidase, beta-glucosidase, has been shown to correlate negatively with the expression of a host beta-glucosidase (Vysotskaya et al. 2015). When we checked whether an ant host alpha-glucosidase was present among the interaction partners of our cestode *lysosomal alpha-glucosidase*, we not only found this, but were also able to show a correlation of their expressions with a linear model ($p = 0.03$ $r=0.113$; Fig. S3). Analysis of GO terms of the interaction partners of *lysosomal alpha-glucosidase* revealed the term *regulation of cellular protein metabolism* with 174 genes. This metabolic pathway is intertwined with glucose metabolism and glycolysis (Carthew, 2022) and may indicate that the cestode interferes with the glucose metabolism of its host. This is also illustrated by the term *response to osmotic stress* (12 genes), which can be linked to altered glucose concentrations (Albertyn et al. 1994). Alpha-glucosidases can also influence neuromuscular functions and a deficiency can cause motor and respiratory disorders, known as Pompe disease in vertebrates (Colella et al. 2020). Therefore, it is perhaps not so surprising that the most common GO term is *neuronal projection development* with

106 genes. Pompe disease decreases glycogen concentration in projection neurons (Sidman et al. 2009) and it is therefore conceivable that alterations in host glycogen regulation by the cestodes alpha-glucosidase is one reason why the inactive cestode-infected workers exhibit Pompe-like symptoms. Finally, we found a GO term associated with cuticle development, i.e. *chitin-based embryonic cuticle biosynthetic process*, which maybe related as chitin-containing materials can serve as inhibitors of alpha-glucosidase (Nguyen & Wang, 2017). Infected ants show a reduced sclerotization of the cuticle, which takes place during the pupal stage.

We also obtained the GO terms for host interaction partners of six commonly secreted cestode proteins that were not annotated (Hartke et al. 2023). Four of these genes were in the dark orange module - the second, third, twelfth and thirteenth most abundant parasitic protein in the ant haemolymph - and two were in the turquoise module - the fourth and sixth most abundant (Fig. 3f). The second most abundant gene has 97 interaction partners and these three significant GO terms, all of which based on two genes (Fig. 3f). One term is *SMAD protein signal transduction*, a signalling pathway involved in muscle hypertrophy and a negative regulator of muscle growth (Nikooie et al. 2020). This term co-occurs with the positive regulation of myocardial hypertrophy. When we examined the other candidates in the dark orange module, we found that these cestode genes also strongly interact with genes of the SMAD signalling pathway (13th most abundant protein) and with other muscle functions such as *neuromuscular processes controlling posture*, with *positive regulation of cardiac muscle hypertrophy* and *adult walking behaviour* (12th most abundant protein). In view of the fact that infected ants suffer from muscular dystrophy (Feldmeyer et al. 2016) and are much less active (Beros et al. 2015), these cestode genes could indeed be involved in the regulation of muscular activity of the host. We also found two immune functions in these interacting host genes *melanin biosynthetic process*, which is involved in the encapsulation of foreign substances (Gillespie et al. 1997) and *positive regulation of lamellocyte differentiation,* lamellocytes being key insect immune cells (Lavine & Strand, 2002). We detected high coherence in the GO terms among the interaction partners of the fourth and sixth most abundant cestode proteins in the ant haemolymph from the turquoise module, both of which interact with genes enriched for *tyrosine catabolism* and *L-phenylalanine catabolism*. Phenylalanine catabolism plays an important role in the encapsulation of malaria in mosquitoes. When phenylalanine catabolism is disrupted by silencing of phenylalanine hydroxylase (Calvo et al. 2008), mosquitoes became unable to encapsulate malaria parasites (Fuchs et al. 2014). The expression of the fourth most abundant protein is associated with host genes involved in lipogenesis, of which we find ten GO terms.

Considering that the fat body is the most important immune organ in insects (Hoffmann & Reichhart, 2002), fat metabolism and immunity are likely to be closely intertwined. Although we did not find specific immune functions in our GO terms besides *L-phenylalanine catabolism* and *response to cobalt ions involved in detoxification*, genes involved in lipogenesis might shed light on how the immune system of infected ants is altered. For example, lipogenesis is suppressed by the Toll signalling pathway in times of immune stress in *Drosophila* (Diangelo et al. 2009), and lipids themselves were shown to play a role in the immune responses against fungal parasites (Wronska et al. 2023). Therefore, these cestode genes may modify host immunity via targeting lipogenesis.

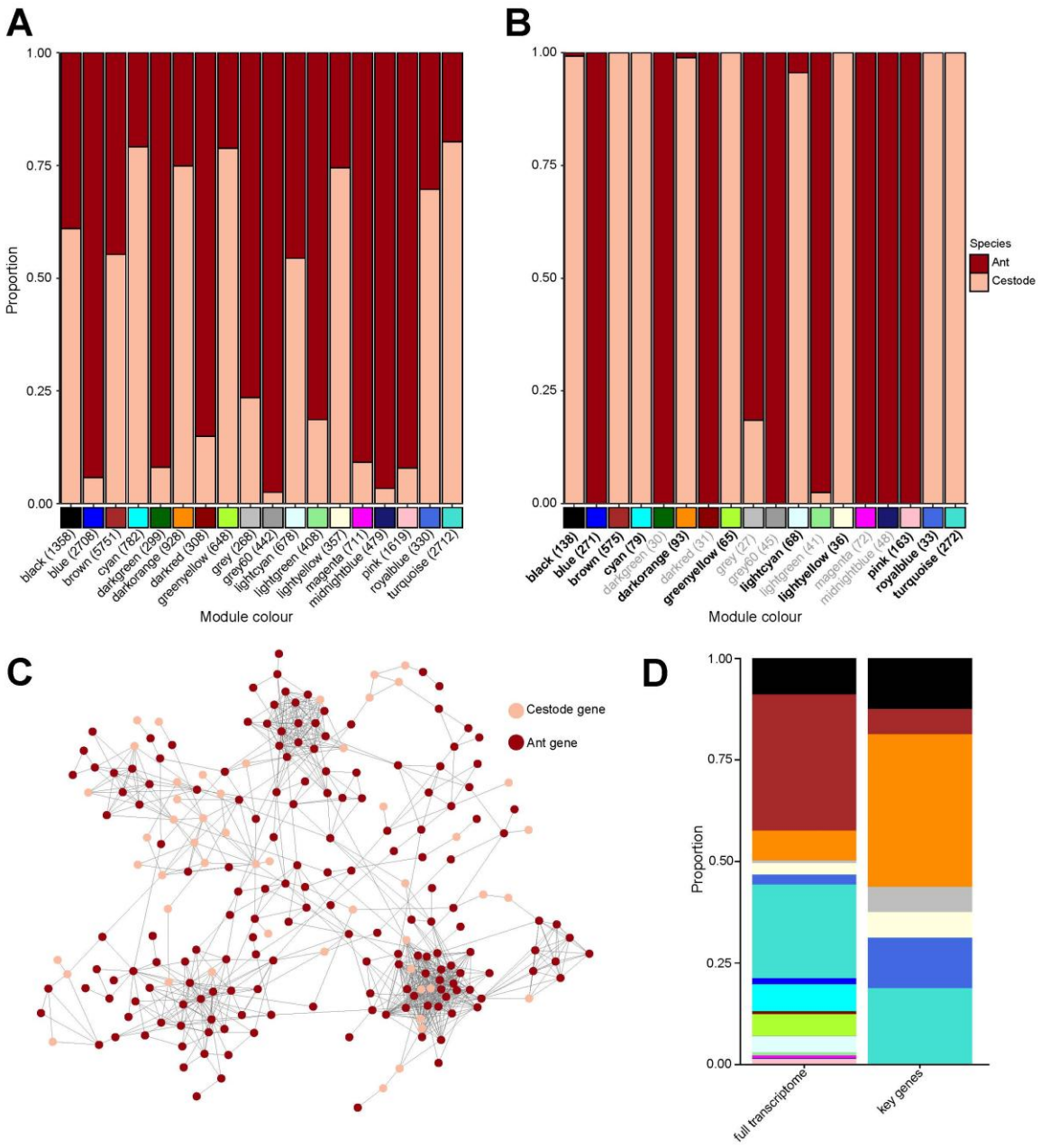
A
B
Proportion
1.00
0.75
0.50
0.25
0.00
Species
Ant
Cestode
black (1358)
blue (2708)
brown (5751)
cyan (782)
darkgreen (299)
darkorange (928)
darkred (308)
greenyellow (648)
grey (268)
grey60 (442)
lightcyan (678)
lightgreen (408)
lightyellow (357)
magenta (711)
midnightblue (479)
pink (1619)
royalblue (330)
turquoise (2712)
black (138)
blue (271)
brown (575)
cyan (79)
darkgreen (30)
darkorange (93)
darkred (31)
greenyellow (65)
grey (27)
grey60 (45)
lightcyan (68)
lightgreen (41)
lightyellow (36)
magenta (72)
midnightblue (48)
pink (163)
royalblue (33)
turquoise (272)
Module colour
Module colour
C
Cestode gene
Ant gene
D
Proportion
full transcriptome
key genes
E
resp. to elec. stim. involved in reg. of muscle adap.
peptidyl-serine dephosphorylation
centriole replication
viral entry into host cell
cation transmem. transport
pos. reg. of calcium ion-dep. exocytosis
G protein-coupled receptor sig. pathway
mating
resp. to elec. stim. involved in reg. of muscle adap.
iodide transport
ADP transport
ATP transport
reg. of start site selec. at RNApol II promoter
peptidyl-threonine dephosph.
cellular resp. to leucine starvation
peptidyl-serine dephosph.
reg. of postsynaptic membrane potential
cell junction assembly
proteasome-med. ubiquitin-dep. protein catabolic proc.
inductive cell migration
neg. reg. of DNA endoreduplication
termination of RNApol II transcr.
chondroitin sulfate biosynth. proc.
reg. of fibroblast proliferation
reg. of NIK/NF-kappaB signaling
chitin-based embryonic cuticle biosynth. proc.
DNA double-strand break processing
male meiosis cytokinesis
artery development
neg. reg. of JNK cascade
resp. to osmotic stress
endocytic recycling
golgi-plasma membr. protein transp.
I-kappaB kinase/NF-kappaB sign.
mitotic spindle assembly
protein homooligomerization
membrane fusion
biological proc. involved in symbiotic int.
reproductive structure development
reg. of establishment of protein localization
neuron projection dev.
reg. of cellular protein metabolic proc.
Superoxide dismutase
Disulfide isomerase
Thioredoxin peroxidase
Alpha-glucosidase
0 10 20 30 40 50 100 150
Number of significant genes
F
neg. reg. of lamellocyte differentiation
pos. reg. of cardiac muscle hypertrophy
SMAD protein signal transduct.
pos. reg. of calcium ion−dep. exocytosis
succinate transmembrane transport
sperm ejaculation
pos. reg. of cholesterol esterification
tarsal gland develop.
linoleic acid metabolic proc.
monounsat. fatty acid biosynth. proc.
cuticle hydrocarbon biosynth. proc.
temperature compens. of circadian clock
pos. reg. of uterine smooth muscle contraction
long-chain fatty-acyl-CoA biosynth. proc.
resp. to cobalt ion
alpha-linolenic acid metabolic proc.
pos. reg. of sensory pain perception
male germ-line stem cell asymm. division
monounsat. fatty acid elongation
polyunsat. fatty acid elongation
saturated fatty acid elongation
cloaca development
tyrosine catabolic proc.
L-phenylalanine catabolic proc.
triglyceride biosynth. proc.
ommatidial rotation
reg. of establish. of planar polarity
fatty−acyl-CoA biosynth. proc.
tyrosine catabolic proc.
L−phenylalanine catabolic proc.
melanin biosynth. proc.
neuromusc. process controlling posture
pos. reg. of cardiac muscle hypertrophy
adult walking behavior
SMAD protein signal transduc.
2nd most expressed
3rd most expressed
4th most expressed
6th most expressed
12th most expressed
13th most expressed
0 2 4 6
Number of significant genes

Figure 3 Results of the interactome analysis. A. WGCNA modules and the representation of genes within these modules per species, pink is for cestode genes, brown for ant genes. Module size is mentioned with the module names in the x-axis. **B.** Hub gene representation for each module, with the same colour scheme as 3a. The number of hub genes is mentioned with the module names. **C.** All interactions within the grey module, each dot represents a single gene within the module and each node represents significant correlation between genes. Distance does not represent stronger correlation. Pink dots are cestode genes, brown dots are ant genes. **D.** Gene module membership of all cestode genes (left) and gene module membership of the 19 most commonly found cestode genes in the secretome according to Hartke et al. (2023). **E.** GO enrichment of ant interaction partners of annotated common cestode genes in the ant haemolymph, colour represents each individual gene. **F.** GO enrichment of ant interaction partners of unannotated common cestode genes where colour represents interaction partners of each individual gene, the order of commonality is mentioned to the right of the bars.

Discussion

The influence of infected worker prevalence and colony size on host and parasite

As infected workers are often even better cared for than the queen (Beros et al. 2021), we expected that infected workers, their nestmates and the parasites themselves would become stressed with increasing prevalence of infected ants in the colony and that this would be reflected in their transcriptional activity. This is indeed what we mostly found, albeit the strength of the response and the genes and pathways targeted varied between these groups.

Infected ants altered the expression of genes involved in lipogenesis pathways which mediate starvation response (Whitehouse & Tisdale, 2001). Also 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, further 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). Considering the directionality of this pathway is being indicated with the GO term we can state that this pathway is being upregulated in the lowly infected colonies, it would mean that this immune reaction is weakened in ants from highly infected colonies. Perhaps it is starvation stress that these ants have to deal with which weakens potential immune reactions to parasites or it is the fact that parasites are generally more virulent in heavily infected colonies due to a higher need to take resources from a starving host. The number of individual genes seems to suggest something similar with a starvation signal being present in several ones. The parasite gene expression data seems to suggest the same as the DEGs in response to prevalence are possibly involved in parasite-host communication. Cestodes are covered by a layer of mucin within their hosts which protects them from the host's immune reaction. Yet this mucin also contains proteins that are capable of forcing dendrite maturation (Casaravilla et al. 2014). This process seems to also inhibit the immune reaction of the host, so that a neuro secretory pathway is heavily involved in parasite-host interactions (Biserova et al. 2023), which might help explain the presence of the other two neurological processes. Besides,

neurological pathways are often being used by gut-based parasites for extraction of resources using the properties of the enteric nervous system (Halliez & Buret, 2015). Surprisingly however we did not find any indications through gene functions that cestodes from heavily infected colonies are suffering from starvation. Therefore it is possible that the cestode might receive sufficient resources by more intensely taking them from the host. This phenomenon has been reported for nematodes where starvation in the host caused a slight increase in lipid content in the parasite (Beames et al. 1967; Smith et al. 1996). The cestode might thus extract lipids from its host which would especially explain why we found negative regulation of fatty acid transport in the downregulated genes with prevalence. Confirmation for these processes could come from the analysis of lipid contents of hosts and parasites in response the ratio of infected workers in a colony.

As for the uninfected ants we would have expected more stress and higher risk of starvation in high prevalence colonies as well. We identified multiple stress reactions especially to that towards oxidative stress with the response to carbon monoxide and response to aluminium ion being the most obvious. This would have multiple explanations considering oxidative stress is caused by a large number of factors. Firstly it could be that uninfected workers have to move more, considering heavy exercise tends to cause oxidative stress (McArdle & Jackson, 2000). This might also explain the shorter lifespan of uninfected workers in infected colonies (Beros et al. 2021) and we would expect this to decrease in more heavily infected colonies for infected ants. Another explanation for the higher oxidative stress could also be starvation (Morales et al. 2004) since uninfected ants obviously have to feed more mouths and would have less food available to themselves. A low food intake can actually increase lifespan in insects and other animals (Hwangbo et al. 2020). In theory this is not likely to be the case as we fed and watered our colonies ad libitum, however, preliminary analysis showed that survival of uninfected ants decreased with the prevalence of infected workers (Rognet & Kokko, pers. inform.). A final explanation of the higher oxidative stress in uninfected ants might be that they have a more active immune system. When nestmates are infected with fungal parasites, ants are known to interact with each other when a nestmate is infected, creating an immune reaction in uninfected nestmates (Konrad et al. 2012). Perhaps infected individuals give off the signal that they are infected which puts the uninfected nestmates' immune system on high alert. This also partially explains the presence of immune genes within our uninfected dataset. We further found two genes of interest in the uninfected dataset which were either buffered in their gene expression or amplified based on colony size. The buffered gene was phosphoinositide 3-kinase regulatory subunit 4-like, which is involved in autophagy (Thoresen et al. 2010). Although we only observe an effect here of one gene, it does give us a clue as to how colony size plays a role in buffering the negative effects that uninfected ants experience as a result of infected colony members. This is in correspondence to previous results that found that the colony can buffer the effects of infection (Scharf et al. 2012). The gene we uncovered whose effect is amplified by colony size was pheromone-binding protein Gp-9-like, which is a selfish gene heavily selected for in ant colonies, specifically in who carries it (Krieger & Ross, 2005). Whether this gene has the same level of selection in *Temnothorax nylanderi* is unknown, though it has shown up in other gene expression datasets (Stoldt et al. 2024). Differential expression of this gene might be linked to the recognition of nestmates and its expression being amplified by colony size and parasite prevalence might hint at the communication between infected and uninfected individuals.

Cestode transcriptomes were more strongly affected by colony size than infected worker prevalence, which indicates that the social organisation is important for this parasite. Colony sizes lead to altered lipid phosphorylation in cestodes. As we have previously discussed, we suspect that the lipid intake of parasites from highly infected colonies is different due to the probable food intake of their host. Having taken into account the effect of prevalence in our model, it would seem that colony size alone also affects the food intake ability of *A. brevis*, or more specifically, how often the host is fed. This likely has to do with the fact that the productivity of the colony is negatively affected by colony size (Karsai & Wenzel, 1998; Michener, 1964; Naug & Wenzel, 2006). This would mean that the food intake is likely lower for infected ants in larger colonies to a degree that it does not affect them, considering the fact that we found only two genes that are affected by colony size in infected ants, while the cestode does need to live on reserves. The strongest effect of colony size alone however was in uninfected ants. Many of the genes affected by colony size in this group seem to have neurological functions, which we interpret as homeostatic due to the fact that we used the fat body rather than the brain. This combined with the fact that we also found specific hormonal functions in this dataset like positive regulation of insulin secretion and that some of the neurological functions are specific to development and growth rather than signal transduction. Aside from that, the fat body and the brain are in constant communication and signal transduction is not entirely out of left-field in the fat body (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 behaviour and locomotion in insects (Erion & Sehgal, 2013). Finally, growth and immunity are also influenced by colony size, this is contrary to previous results however where it was found that colony size is not a predictor for investment in antimicrobials (Penick et al. 2018).

Parasite-host-co-expression analysis

Through our novel usage of the WGCNA in a joint analysis we have been able to gain insights in how the expression parasite and host genes are intertwined. Generally we found that the expression of cestode genes is linked to far more genes than host genes are. In six modules ant genes were more likely to interact with cestode genes than with host genes. In each of these modules cestode genes were more likely to interact with cestode genes. In order to achieve that, the expression of ant genes is less linked to other genes than the expression of cestode genes. This is effect also becomes apparent in the hub genes of these modules, where each of the modules where ant genes were more likely to interact with cestode genes, cestode genes utterly dominated the hub gene representation. There might be a very simple explanation where *A. brevis* has mainly adapted to *T. nylanderi* and does not need to commit much of its gene expression to other factors other than the host. Therefore its interaction with the host comprises of a large part of the gene expression. *T. nylanderi* on the contrary interacts with nestmates and the biotic and abiotic environment. To serve as an example, we imagine a situation where the host is being challenged by a bacterium. In order to fend off the threat the host will have to activate its immune system, altering its gene expression in the fat body. However, besides the immune system, the rest of the host's gene expression should be minimally impacted. For the cestode, a host immune challenge would mean that it possibly needs to activate its own immune

system in order to prevent hyperparasitism (Parratt & Laine, 2018; Pirovino et al. 2023), to regulate the host's immune system upon (Hernandez et al. 2013) and adapt to a possibly more hostile environment caused by the immune response (Mideo, 2009). This would mean the co-expression between the parasite's stress responses, immune system, parasite-host communication with only the host's immune system.

This does bring up a possible problem with parasite-host co-expression analysis, we do not only investigate genes that interact but also genes whose expression is indirectly influenced by the expression of others, and correlation does not equal causation. The real value of this type of analysis lays in the study of individual genes, which we have done with the commonly expressed parasite genes in the ant haemolymph. For the annotated genes we could clearly see that the function of these genes correlated biological processes of the ant that were either similar or are prone to be influenced by this gene in parasites.

Firstly we highlight the oxidative stress genes of superoxide dismutase and thioredoxin peroxidase. Here we found the expression between the two to be very interactive, they were in the same cluster and superoxide dismutase interacted with all but one gene that thioredoxin peroxidase also interacted with. This is entirely expected as previous studies have shown superoxide dismutase and thioredoxin peroxidase to be part of the same pathway in both systems without parasites (Pedrajas et al. 2010) and in cestode parasites (Salinas et al. 1998). As for the genes they correlate with, it is clear that these genes are not necessarily involved in communication with the host but rather in the defence against the oxidative stress of the host. It has already been theorized that especially thioredoxin peroxidase is mainly secreted into the host by parasites in order to modulate the oxidative stress that is naturally created in the host through aerobic metabolism (Halliwell & Gutteridge, 1989; Salinas et al. 1998). This is confirmed by the fact that we find gene functions mainly involved with natural metabolism and the correlation with other genes that protect the host from oxidative stress. Whether this protects the host from oxidative stress, extending the lifespan remains unknown, but considering in other parasite-host systems some of the most commonly expressed genes are also involved in oxidative stress response (Chehayeb et al. 2014), it would seem that the host lifespan is extended through different means. What does become clear from this data however is that *A. brevis* appears to prefer an aerobic environment in the host while Platyhelminthes are known to be capable of adapting to an anaerobic environment in different life stages (Martínez-González et al. 2022). For disulfide isomerase we mainly found that the functions of its interaction partner are linked to secretion which concurs with common functions of disulfide isomerases in parasites and multiple other systems (Naguleswaran et al. 2005; Stolf et al. 2011). Once again serving as confirmation that our methods are conform with previous results gained through other means. We can also show this through the final annotated gene, alpha-glucosidase. Firstly in the sense that, similar to beta-glucosidase in previous research (Vysotskaya et al. 2015), we find a negative correlation between it and host alpha-glucosidase. Furthermore we found interaction partners to be involved with glucose metabolism. Alpha-glucosidases are enzymes that are involved in the breakdown of polymeric glucose variants such as starch or glycogen to monomers (Krasikov et al. 2001). This gene is therefore a likely candidate for genes that are involved in the food intake of the cestode, in which glucose uptake plays an important role in cestodes in general (Kashiide et al. 2018). This would be in contrast to the host, which, in an infected state should display lower

levels of glycogen, however, it has been shown that metacestode infected beetles in fact have higher glycogen levels (Novak et al. 1993). This could also be a reaction instead to cestode glucosidase in order to maintain stable glycogen levels.

Finally we will discuss the functions of host genes whose expression is linked to as yet unannotated proteins secreted into the haemolymph of the ant. The four genes in the dark orange module could be involved in the manipulation of the host's behaviour and morphology. Here we found host genes with functions including development of muscles and the SMAD pathway, so that these cestode genes could affect muscle development of the host (Feldmeyer et al. 2016). This might be difficult to test experimentally, as even their knockdown might not immediately result in more active behaviour from the host, as host's muscle development is likely inhibited during its larval stages. There is however a chance that interference of this cluster of genes would result in more active behaviour from infected ants considering these genes also interact with pathways involved with walking behaviour. The reason that we find this GO term in the fat body would be that the cestode cannot specifically target the ant's brain with these protein products, which would also explain the high quantity of these genes in the haemolymph (Hartke et al. 2023). The fact that these genes are differentially expressed in the fat body could be considered a side effect, or vice versa, that the walking behaviour is inhibited due to the interference of the cestode with muscle development. Finally, the two cestode genes we found in the turquoise module which are seemingly involved in the inhibition of the immune reaction, with the possible side effect that it inhibits the cuticle sclerotization as well. This mainly due to the fact that we found a large number of genes involved with lipogenesis and a number of more direct immune genes. Of course, this can also be as a reaction to the host's immune reaction instead, though previous studies on this system suggest that the parasite is indeed capable of inhibiting the immune response against it (Sistermans et al. in prep.). We suggest that future experiments investigate this pathway specifically for the host, where we believe that the silencing of key genes within this pathway in larvae would result in the underdevelopment of the cuticle. Furthermore, in adult infected ants the silencing of these two candidate genes could result in an immune reaction towards the cestode. We would not necessarily expect the cestode to die from this as it has been shown to be able to adapt when the ant's immune system is activated and high parasite load seems to increase an immune reaction while cestodes instead invest on immune avoidance (Sistermans et al. 2023; Sistermans et al. in prep.).

Conclusion

In this study, we were able to demonstrate the effects of prevalence at the colony level, which affect not only the individual but also its nestmates that interact closely with the host and the parasite itself. These effects are not often discussed in the context of parasitism, and although group integration is not as great in most organisms as in eusocial insects, communication between healthy and infected individuals can play an important role in mitigating fitness losses caused by parasitism. The influence of group size on the gene expression of healthy ants and even on the parasite itself also points to the importance of the social group.

Furthermore, using WGCNA, we have shown that parasite gene expression is closely linked to host genes. In doing so, we have identified the relationships involved in parasite-host communication that have the potential to further improve our understanding of this parasite-host interaction. However, we also encourage others to apply these methods to other, understudied parasite-host systems to explore key genes involved in these interactions. In this way, we were able to shed light on which specific host genes and functions are linked to the expression of candidate parasite genes, the proteins of which are known to be secreted into the host. Finally, we believe that this method can also be useful for other types of gene expression data, such as communication between different tissues and other forms of symbiosis.

Acknowledgements

We would like to thank [redacted] for their help with RNA isolations, [redacted] for the lively discussions about the results and [redacted] for teaching the author how to dissect ants.

Supplementary materials

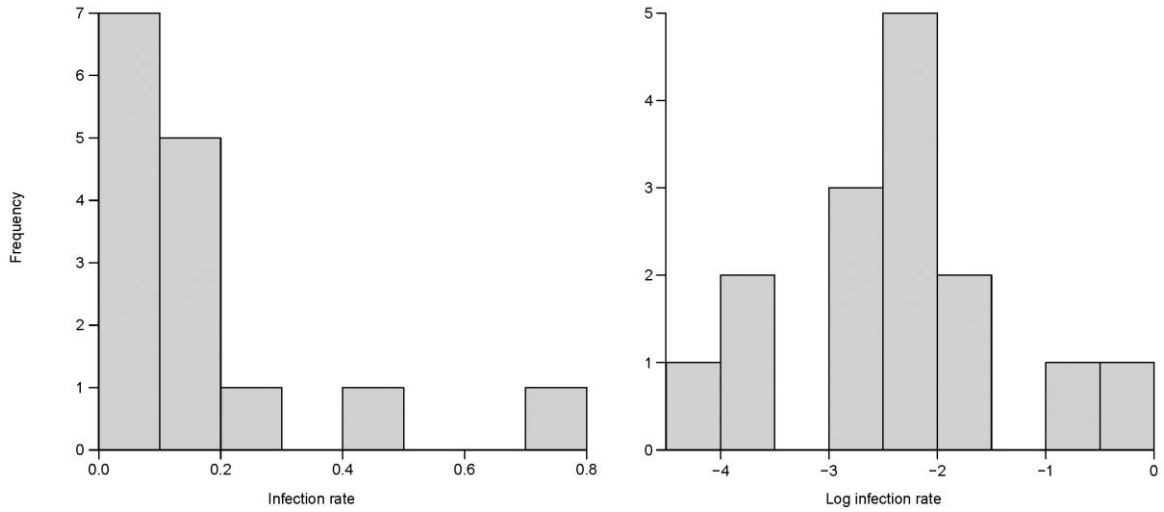

Figure S1 Histograms of parasite prevalence (right) and the log-transformed parasite prevalence (left).

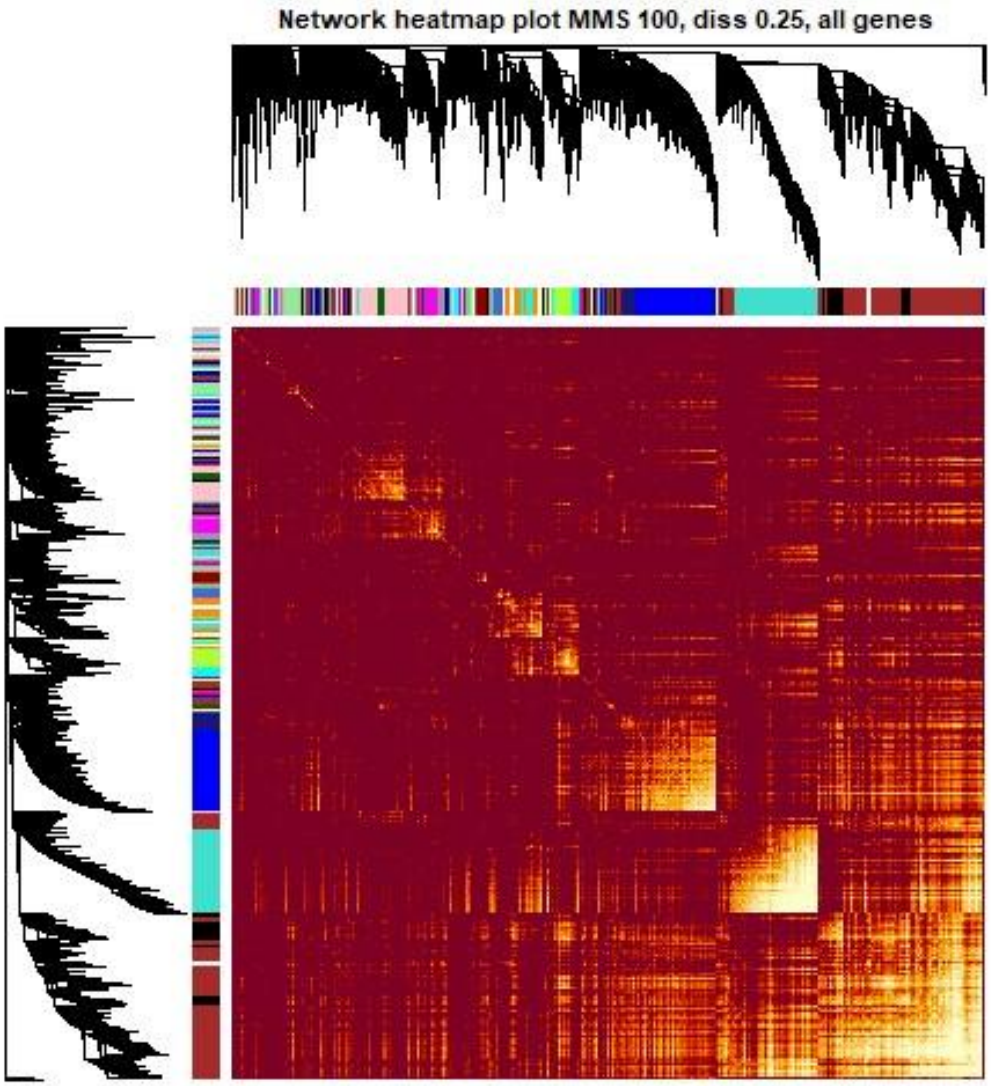

Figure S2 TOM plot of the merged gene count matrices of both infected ants and their cestodes. The colours representing the constructed modules roughly aligning with the highest correlations in the heatmap.

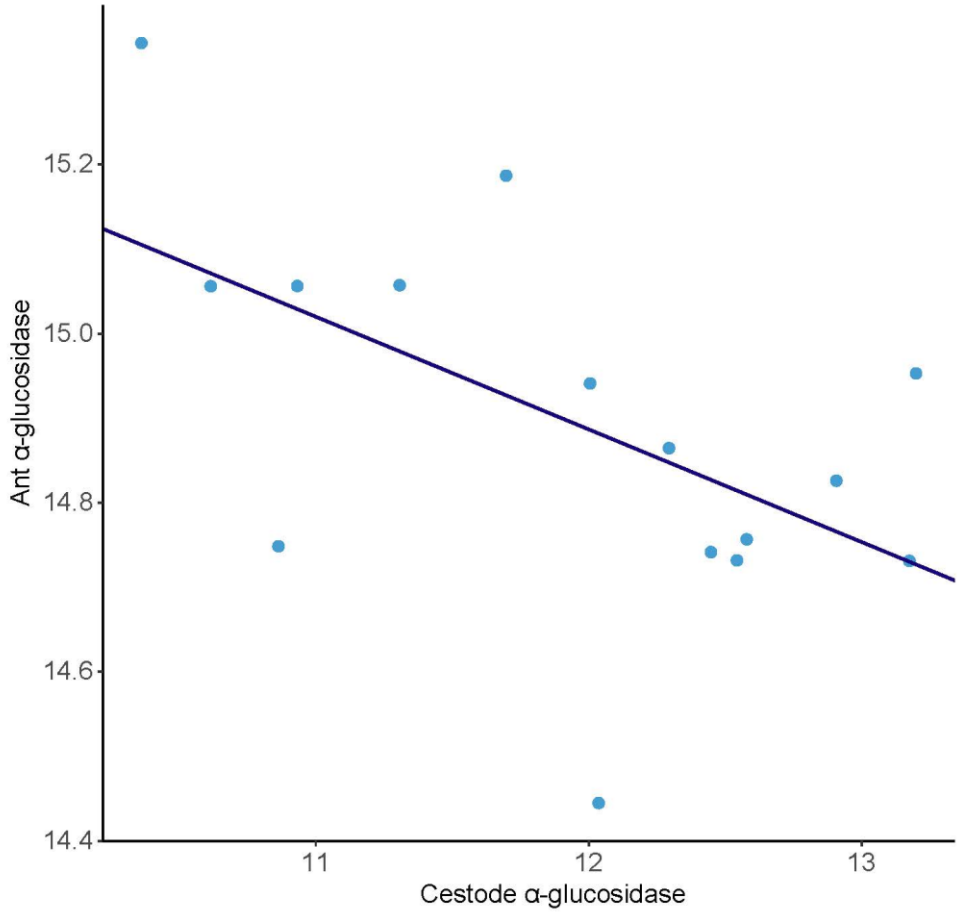

Figure S3 Cestode alpha-glucosidase and ant alpha-glucosidase plotted against each other after variance stabilizing transformation. Here we found a negative correlation with an R value of -0.113 and an intercept of 16.5 (p=0.032).

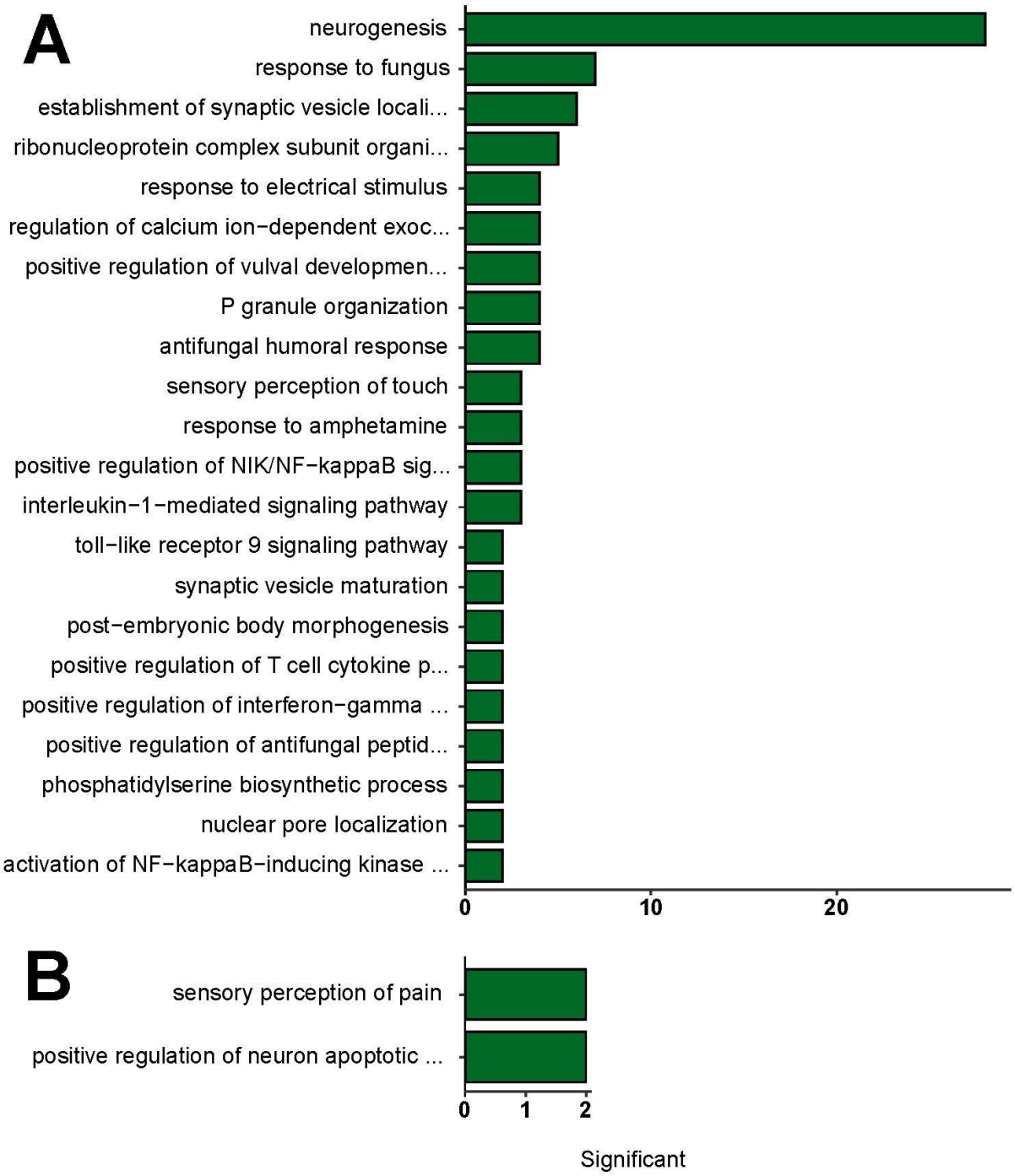

Figure S4 GO terms for the DEGs for interaction between infection and colony size for A. uninfected ants and B. cestodes.

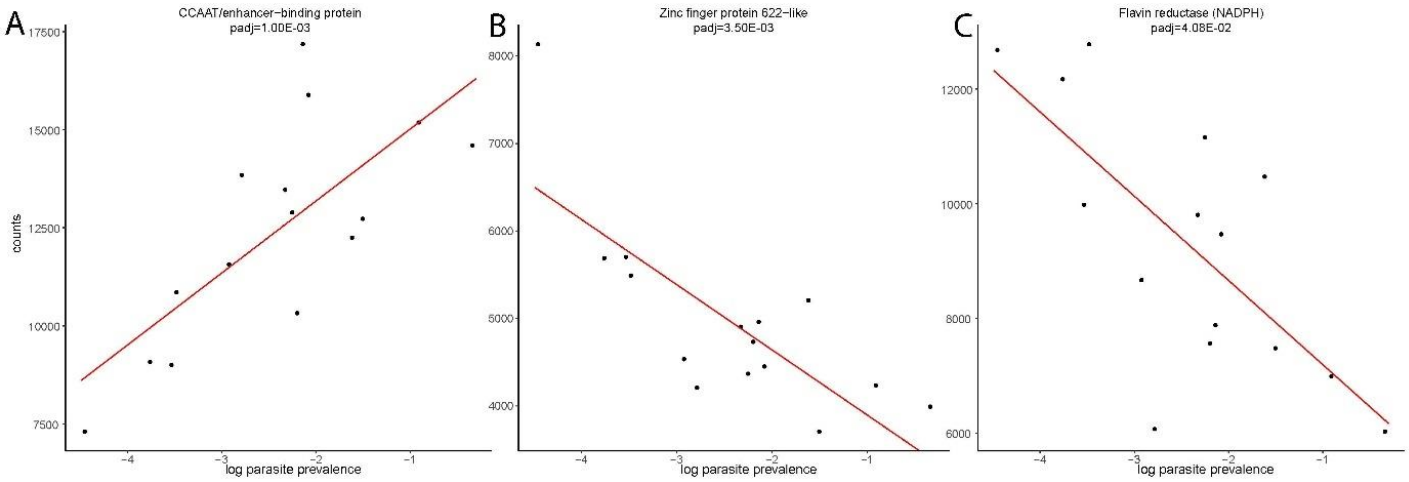

Figure S5 The normalized gene counts of three genes significantly differentially expressed for the parasite infected ants plotted against the logarithm of the parasite rate. A. CCAAT/enhancer-binding protein (r=1835, padj=1.00E-03), B. Zinc finger protein 622-like (r=-745.3; padj=3.50E-03) and C. Flavin reductase (NADPH) (r=-1470; padj=4.08E-02).

Table S1 overview of all the samples used in this paper with all information used for the analysis.

Colony	Infected sample name	Uninfected sample name	Sequencing year infected	Sequencing year uninfected	Cestode sample name	Parasite rate	Cestode number	Colony size
A	A6F	A33F	2020	2022	A6P	0.403	8	77
B	-	B6F	-	2020	-	0.062	-	65
C	C1F	C17F	2020	2022	C1P	0.062	5	44
D	D5F	D17F	2020	2022	D5P	0.098	7	164
F	F2F	-	2020	-	F2P	0.031	8	65
G	G1F	GXF	2020	2020	G1P	0.054	8	93
H	H1F	H5F	2020	2022	H1P	0.023	10	129
J	J8F	J17F	2020	2022	J8P	0.222	7	72
K	K4F	K6F	2020	2022	K4P	0.029	6	137
L	L18F	L141F	2020	2022	L18P	0.712	7	177
M	M16F	M21F	2020	2022	M16P	0.198	6	121
O	O1F	O2F	2020	2022	O1P	0.012	7	86
P	P2F	P3F	2020	2022	P2P	0.125	5	16
Q	Q4F	Q10F	2020	2022	Q4P	0.111	8	72
S	S3F	S5F	2020	2020	S3P	0.118	13	17
T	T1F	T11F	2020	2020	T1P	0.105	9	76

General discussion

Main findings

Parasites shape life on Earth today. At the large scale, we can recognise this in the flow of energy throughout ecosystems (Kuris et al. 2008). But also in the phenotypic traits of organisms that are formed by the selection pressure exerted by parasites (Poulin et al. 2020). And as has become clear in the research projects of my dissertation, it can also be visualised at the molecular level.

In **Chapter 1**, we analysed the effects of parasite load on parasite and host. Parasite load can be described here as the number of parasites per host, which can have possible consequences for the immune system, host size and behaviour (Pelletier & Festa-bianchet, 2004). The parasite load seems to have a strong effect on the host, as it causes more stress and alters the innate immune response. This is mirrored to some extent in the parasite, where we observe a differential reaction to the immune response in the form of a large number of serine proteases involved in evading the immune system. We also found a differential response to stress and starvation, which we would have expected with a higher parasite load, as more cestodes in a single host would mean that fewer resources are available. We also found several processes that are not affected by parasite load but that are regulated differently in infected and uninfected ants. Examples of these processes influenced by infection status alone are the innate immune response and energy metabolism.

In **Chapter 2**, we investigated the immune response of *Temnothorax nylanderi* to both *Anomotaenia brevis* infection and a sham bacterial challenge. Moreover, we questioned whether the effects of both immune stressors act independently or whether they interact by analysing gene expression, but also how both factors and their interactions affected the gut microbiome. We found that both the immune challenge by bacterial lipopolysaccharides and the cestode infection had profound effects on the immune system that were not interdependent. Thus, the cestode did not weaken the immune system of *T. nylanderi* workers to such an extent that they were no longer able to fight off a bacterial threat. However, genes that altered their expression due to a cestode infection and following a sham bacterial threat, did so in opposite directions. For example, a gene upregulated during cestode infection, was downregulated following the sham bacterial challenge, which could be the result of inhibition of the ant's immune system by the cestode. However, the fact that we found no interaction between bacterial and cestode immune challenges means that when challenged by other pathogens, the immune system still functions to a degree that it can fend off other threats. Presumably, this is advantageous for the cestode, as its host stays alive longer and the cestode does not risk hyperparasitism or competition with other pathogens for resources. Indeed, infected workers have a long lifespan with more than half surviving three years in the lab and individuals being known to get as old as seven years. A functional immune system to fight off other pathogens might therefore be valuable. Meanwhile, we found that the gut microbiome probably plays a minor role in the interaction between *A. brevis* and *T. nylanderi*. We did not find any bacterial groups associated with infection with the cestode or infected colonies, implying that the cestode likely does not affect the host gut microbiome or that certain microbiota do not facilitate or deter *A. brevis* infection. However, we observed a relative increase in intracellular bacteria, particularly *Wolbachia* and *Entomoplasma*, in infected ants. We hypothesise that this is due to an earlier immune response possibly during cestode infection that did not affect intracellular bacteria.

Finally in **chapter 3** we tested whether cestodes exploit the superorganism of a *T. nylanderi* colony and we used a novel method of analysing the parasite-host interactome. For the question on whether

entire colonies of *T. nylanderi* and their traits are affected by cestode infection we took infected ants, uninfected ants and cestodes from colonies with differing rates of infected individuals. We discovered that the prevalence of infected workers has a profound influence on both parasites and host, whether directly infected by the parasite or burdened with caring for infected nestmates. As the proportion of infected workers increased, we found evidence of increasing physiological costs in the hosts and greater nutrient uptake by the parasite. Uninfected ants also showed a stress response to the higher prevalence caused by either starvation or overwork. In the interactome analysis, we learnt that the expression of cestode genes is linked to host genes of specific functions with which they are strongly co-expressed. In studying the interactions of cestode candidate genes, where we have evidence that their proteins are secreted into the host, we identified important processes in the ant transcriptome that are related to phenotypic shifts found in infected ants. These interaction partners may be candidates for subsequent analyses.

In the following sections, I will discuss the results of each chapter as a whole in order to understand the results in the context of the objective of this thesis. By gaining insight into the nature of the interaction between the cestode *A. brevis* and its intermediate ant host *T. nylanderi*, we can find specific phenotypic traits that are manipulated by the parasite or are the result of side effects. In addition, I will further investigate the dynamics of gene expression in this system and find out under which conditions we find similarities and differences.

Parasite influence on the host immune system

Some of the results of the various studies appear at first glance to lead to contradictory conclusions. For example, in **Chapter 1** we discussed that a higher parasite load probably leads to a stronger immune response against *A. brevis*. This was supported by GO terms related to innate immunity in the ant as well as genes involved in immune evasion in the cestode. However, in **Chapter 2** we found very strong evidence that *A. brevis* inhibits at least part of the immune response. However, I believe that this highlights a potential drawback of the parasite's interference with the immune system. As mentioned in **Chapter 2**, the complete inhibition of the host's immune system may seem beneficial to a parasite at first glance, but it also increases the host's susceptibility to other parasites and thus creates more competition for the resident parasite (De Roode et al. 2005; Dumont et al. 2007; Mideo, 2009; Vardo et al. 2007). Therefore, two possible scenarios could explain why the immune reaction is still stronger when the *T. nylanderi* is infected with multiple individuals of *A. brevis.* Firstly, this could be the result of interspecific competition, where different individual parasites can try to get rid of one another through incomplete activation of the immune system. This would make this type of parasite behaviour an example of Hamiltonian spite (Hamilton, 1970). If this is a case of spite it would mean that cestodes residing in *T. nylanderi* are not related. Or at least not the ones in our dataset considering Hamiltonian spite can only be achieved with a negative kinship between competing individuals. Furthermore, this strategy would be risky as immune activation of the host would also make the initiating cestode more vulnerable. Therefore, a more logical conclusion would be that a higher parasite load increases the hosts' immune reaction, which has been shown in multiple pathogen-host systems (Borges et al. 2012; Good et al. 2005; Jong et al. 2006). The opposite could be said as well since higher parasite load has been shown to increase virulence (De Roode et al. 2007), which has mainly been linked to a weaker immune reaction (Cornett & Sorci, 2010), however, we find no evidence of higher virulence upon high parasite load in the phenotype data in **chapter 1**. The combination of these results therefore shows evidence of a trade-off for the cestode when it comes to

inhibiting the immune system, whereby the cestode cannot inhibit the immune system sufficiently to let large numbers of cestodes go undetected. If such a threshold is crossed and the presence of cestodes activates the ants' immune system, they have alternative mechanisms for immune avoidance through the use of serine proteases (Dzik, 2006; Izvekova et al. 2021; Pleass et al. 2000). These mechanisms are likely also in place to avoid the immune response in times of immune stress from a different pathogen, as has been shown in **chapter 2** or in times of cestode crowding as exemplified in **chapter 1**.

Immune response manipulation and side effects

One specific immune process that has been shown in several of my studies is the melanisation pathway, which is part of the encapsulation response, a cellular immune response of insects that is used against pathogens that are too large to be phagocytosed (Nappi & Vas, 1993; Paskewitz & Gorman, 1999; Strand & Pech, 1995). It firstly shows up in **chapter 2** where it appears among the differentially expressed genes upon cestode infection, here we found proteolysis to be among the GO terms in the differentially expressed genes, which is involved in melanisation response (Hoffmann, 1995; Schmidt et al. 2008). It was also among the GO terms in the co-differentially expressed genes between cestode infection and LPS immune challenge. Considering that all these genes were expressed in different directions, this is a clear indication that the melanisation response was inhibited in either cestode-infected ants or LPS immune challenged ants, upon closer inspection of the BLAST, the genes involved with proteolysis unfortunately were either uncharacterized or their function in cuticle formation was unknown. A second indication that melanisation is regulated differently in bacterial and helminthic immune threats is the fact that at least three of the co-differentially expressed immune genes are involved in the Toll signalling pathway, which is closely linked to the melanisation response (Kan et al. 2008; Roh et al. 2009). Especially the involvement of protein Spaetzle, which also shows strong connections to cuticle formation (Kondo et al. 2017). Given that cestodes have the ability to inhibit their host's immune response (Hernandez et al. 2013; Izvekova & Frolova, 2017; Vendelova et al. 2015) and that LPS challenging has been shown to cause an immune response in multiple insect systems (Charles & Killian, 2015; Walderdorff, Laval-Gilly, Bonnefoy, & Falla-Angel, 2018), we can conclude that the melanisation pathway is somewhat inhibited in cestode-infected individuals. This could also explain that cysticercoids in the ant's haemolymph escape melanisation. In **Chapter 3**, we again encountered the melanisation response in the interactome analysis. Here, the gene encoding the twelfth most abundant protein in the cestodes' secretome interacted with ant genes enriched for the melanin biosynthesis process, while another gene in the same module interacted with the L-phenylalanine catabolism process, both of which are involved in the melanisation response to foreign threats (Fuchs et al. 2014; Gillespie et al. 1997). However, when looking at other GO terms in this module, we mainly concluded that these cestode genes most likely have effects on ant behaviour and morphology, especially on the development of muscles in the host. We also found L-phenylalanine catabolism linked to the gene encoding the fourth most secreted protein, which was located in a different module. The interaction partners of the ants were clearly enriched for immune functions and fat body functionality. Therefore, these cestode genes were likely candidates for avoidance or manipulation of the host immune system.

The frequent occurrence of the melanisation reaction in our results can be of interest because the melanisation pathway is also associated with the sclerotization and pigmentation of the insect cuticle (Andersen, 2011). This obviously reminds us of the fact that infected *T. nylanderi* display a soft and less

coloured cuticle (Plateaux, 1972). Therefore, the cuticle of infected ants might be less pigmented due to the suppression of the melanisation reaction by the cestode, especially during the pupal stage when the cuticle is formed. This is possible because cestode infections occur during the larval stage of the ant. There is a possibility that L-phenylalanine is part of this process, as it has been shown to influence the tanning of the cuticle (Arakane et al. 2009). Another reason to suspect that the inhibition of cuticle pigmentation is simply a side effect is by reasoning whether the parasite can potentially gain fitness from an untanned cuticle. The reasoning has always been that the yellow cuticle makes ants easier to spot for the final host or perhaps even look like brood. However, the question might be whether the final host even needs the ants looking like this to be able to spot them. According to Otto Fuhrmann, a parasitologist who has been at the cradle of investigation in *Anomotaenia* species, among other bird cestodes, far before it was even known that *A. brevis* infects ants, had noted that *Anomotaenia* can be found in both woodpeckers and Eurasian jays (Fuhrmann, 1909). Both jays and woodpeckers are known to forage specifically on ant nests in wood or acorns rather than insects that walk free (Clayton et al. 1996; Gorman, 2015). In the case of woodpeckers it has even been reported that their foraging efforts are not detrimental to the colony and they are capable of remembering where ant colonies are located (Leite et al. 2013). It is also likely that birds are perfectly capable of spotting *T. nylanderi* in the wood anyway since most birds are tetrachromatic and are capable of seeing more colour than the average human (Bennett & Théry, 2020). Therefore, the light cuticle colouration, as it clearly differs from the brood colour might even serve as a warning sign for foraging woodpeckers which would lead to the cuticle colouration being a selectively negative extended phenotype of *A. brevis*. This would of course also depend on the negative fitness effects *A. brevis* would have on woodpeckers, if the cestode would be highly virulent in woodpeckers, it's more likely this cestode recognition would be selected for. This is not likely to be the case since cestodes have shown to not affect bird population dynamics much (Harris et al. 2018). Something more likely to negatively impact the fitness of *A. brevis* is that the cuticle underdevelopment might increase the host's susceptibility for other pathogens, since the cuticle is an important first barrier for parasites to overcome (Moret & Moreau, 2012). Overcoming the cuticle is something that *A. brevis* does not have to achieve since *A. brevis* enters the host orally and before cuticle development (Trabalon et al. 2000). This brings us to the main dilemma facing parasites described in **Chapter 2:** While suppression of the immune system may be beneficial to the parasite's ability to survive in the host, it also makes the host more susceptible to other pathogens that could also compromise the parasite's fitness. While it is clear that the host is still able to fend off bacterial threats, deactivating a barrier that prevents other pathogens from entering the host seems downright detrimental unless the encapsulation response is also inhibited. Therefore, it is not surprising that terms referring to the encapsulation response co-occur with terms that could lead to some of the other typical phenotypic traits of infected *T. nylanderi*. One argument in favour of the fitness advantages of a non-sclerotised cuticle is that when eaten by a woodpecker it would be easier for *A. brevis* to leave the body of *T. nylanderi* in the gut and attach to woodpecker gut walls. The cestode only has this one chance to parasitise its final host, if it is excreted before it gets out of its intermediate host, its life ends. However, this would be a unique occurrence of sclerotization inhibition caused by a cestode. Other cestodes are known to infect woodpeckers, including several species of *Raillietina* (Foster et al. 2002; Nawab et al. 2016) which are part of the same order as *A. brevis*. Multiple species are also known to infect insects as an intermediate host (D. G. Baker, 2008), as far as could be determined, however, they do not lead to a change in the sclerotization of the ant and beetle species they infect. At least the fact that the colouration of the

cuticle could lead to a decrease or increase in the fitness of *A. brevis* can be investigated relatively easily by observing the foraging behaviour of woodpeckers to determine whether they actually skip infected nests. If this is the case, there is evidence to suggest that *A. brevis* may inadvertently protect ant nests from predation, possibly leading to a fitness advantage of infected *T. nylanderi* colonies in areas under heavy predation pressure by woodpeckers.

When considering possible negative effects on fitness due to the cuticle of infected *T. nylanderi* forming a poorer barrier against pathogens, the idea of social immunity in ants must also be considered. It could be that this lower immune response is compensated by a higher social immunity. This would mean that colonies with a higher infected worker prevalence have a stronger immune response. In **Chapter 3**, we may have found this in uninfected ants, where they experience a higher oxidative stress response, possibly due to the active immune system. We did not find specific immune responses in infected ants, but this could also be because the social immune response in ants consists mainly of grooming and removing threats from the cuticles of nestmates (Stock et al. 2023) or by distributing antipathogenic substances such as resin (Chapuisat et al. 2007). Therefore, the transcriptomic signal for this specific immune reaction might not be found in the fat body of the ants but rather in the brain. Indeed, workers from infected colonies exhibit a higher rate of self-grooming and allogrooming behaviour, whether they are infected themselves or not (Scharf et al. 2012). This is strengthened by the fact that infected ants receive more social attention than even queens (Beros et al. 2015). Therefore, a possible follow up experiment to investigate this aspect of infected ants can be done by investigating how they receive attention from uninfected nestmates in pathogenically hostile environments.

Finally, we have to discuss other possible side effects, especially after those we found in the interactome analysis in **chapter 3**. As mentioned earlier, genes that seem to have an immune function interacted with cestode genes that also interacted with genes enriched for the development of muscles. This could be yet another signal of a clear phenotypic characteristic in infected ants, namely the muscle dystrophia (Feldmeyer et al. 2016). The muscle underdevelopment of infected ants is probably one of the easiest examples of clearly benefiting parasite fitness. Having dissected over 1000 ants over the course of my PhD, one thing that became clear is how easy it was to pick up an infected ant. They display extremely sluggish behaviour and seem to have trouble running away from forceps. This is of course confirmed by statistics in previous research efforts on this system (Trabalon et al. 2000). The fact that it becomes so easy to pick them up surely makes it easier for infected ants to be preyed on by woodpeckers and therefore one might assume that this extended phenotype can be regarded not as a side effect but an active manipulation. According to what we found mentioned earlier however, the immune reaction and muscle development might be somewhat connected here. In the end this might tell us a lot about parasite evolution and how characteristics that are positive for parasite fitness might start off as side effects of manipulating other aspects of the host. Sluggish and muscle development in that regard is barely a rare side effect and both have been observed in other parasitized hosts. For sluggish behaviour this includes nematodes in humans (Jasti et al. 2007), *Toxoplasma* in multiple hosts (Gatkowska et al. 2012; Sugden et al. 2016) and cestodes in fish (Dence, 1958; Orr, 1966). Muscle dystrophia meanwhile has been caused by bacteria, viruses, nematodes, trematodes and cestodes (El-Beshbishi et al. 2012; Shin et al. 2023). This might create a slight chicken or the egg story and leads us to ask which possible manipulation mechanism, the immune inhibition or the muscle dystrophia, came first, if this can even be stated. This shows that the influence that

parasites can have on their host's phenotype might be multifaceted and parasites can possibly be very efficient when it comes to manipulation, since the disruption of a single pathway might kill two birds with one stone.

Oxidative stress and ageing

Something that can also be possibly explained as a co-occurring phenotypic characteristic of the host might be the long lifespan of the host (Beros et al. 2021) together with the oxidative stress response. Previous efforts have already found evidence for this co-occurance, firstly in the shape of infected ants having a differential oxidative stress regulation than uninfected ants through the expression of cytochrome c oxidase (Stoldt et al. 2021), whose disfunction causes a reduction of reactive oxygen species (Srinivasan & Avadhani, 2012). Later evidence also leads us to the fact that cestodes release antioxidants into the host (Hartke et al. 2023), among which superoxide dismutase (Yang et al. 2007) and thioredoxin peroxidase (Wang et al. 2019). Meanwhile in our datasets we also find multiple instances of oxidative stress, firstly in **chapter 1** where we found oxidative stress response to be differentially regulated between lowly infected ant highly infected ants. In **chapter 2** this was mainly the case in LPS immune challenged ants, where we found the COVID-19 KEGG pathway in cestode infected ants which is involved in the creation of oxidative stress upon viral infections (Amor & Blanco, 2020), but also in GO terms for both LPS immune challenge and cestode infection with glutathione metabolic process, which instead protects against severe oxidative stress (Maher, 2005). We also found the latter to be among the GO terms that were regulated in opposite directions in LPS challenged and cestode infected ants. Finally in **chapter 3**, both infected and uninfected ants experience different degrees of oxidative stress as a result of colony parasite prevalence.

Ageing and oxidative stress are highly linked (Finkel & Holbrook, 2000; Lenaz, 1998; Luo et al. 2020). This has to do with the mitochondrial free radical theory of ageing, which states that ageing is caused by the accumulation of unrepaired oxidative damage (Gruber et al. 2011; Harman, 2002; Linnane et al. 1989; Miquel et al. 1980). In that case we can state that oxidative stress causes ageing and therefore that processes that cause oxidative stress might speed up ageing. This is certainly the case for the immune system which, as mentioned earlier, can create oxidative stress in order to fend of pathogenic threats. This can be observed with one of the differentially expressed genes we found in **chapter 1**, dual oxidase 2, whose upregulation has been shown to cause UVB-induced ageing (Xiao et al. 2021). The inflammation response has also shown to be able to contribute to ageing where a constant background inflammation response in older people has shown to contribute to age-related pathologies (Alberro et al. 2021; Pawelec et al. 2023). Considering infected ants do not seem to have a strong immune reaction to the cestode as described in **chapter 2** due to cestode immune interference, this can already serve as a factor to slow down ageing in the ant. This can also be said for natural metabolism as explained by the production of free radicals by mitochondria, but in this regard possibly directly regulated by the cestode as it secretes antioxidant enzymes into the host. As described in **chapter 3**, these antioxidant enzymes were especially co-expressed with host-metabolic pathways and seem to be a means of mitigating the oxidative stress caused by metabolism. This is strongly reminiscent to another cestode-host system between rats and the cestode *Hymenolepsis diminuta*, where an important part of its proteome is involved with detoxification response and oxidative stress (Bień et al. 2016). Unfortunately, it hasn't been researched in this tapeworm species yet with its intermediate host, the beetle *Tenebrio molitor*, as the cestode has been known to increase the lifespan of the beetle (Hurd et al. 2001). Furthermore, cestodes are also known to protect their host

from oxidative stress caused by external factors such as heavy metals in the environment (Sánchez et al. 2016). Ironically, the parasite itself might even be one of these external factors when regarding the uninfected ants in the colony, since we've shown in **chapter 3** that uninfected nestmates seem to deal with several stressors depending on how heavily saturated the colony is with infected ants. Finally, infected ants might also deal with this depending on their parasite load as has been described in **chapter 1**, since we discovered an upregulation of dual oxidase 2 with high parasite load, whose upregulation, as mentioned earlier, can increase UVB induced ageing (Xiao et al. 2021). With all that, it might seem that the cestode's primary goal with this is to regulate oxidative stress in the host while also extending the lifespan, thus, a co-occurring parasite-mediated phenotypic change.

Parasite load exists both on a colony and individual level

When regarding the last section, one cannot help but notice a certain similarity with ants from heavily infected colonies and heavily infected ants. In fact, one might be able to put the similarities in context when interpreting infected ants as parasites within their own colony. As mentioned multiple times, the fitness cost of parasitism seems to mostly be allocated to uninfected nestmates and the colony productivity, since uninfected nestmates live shorter (Beros et al. 2021) and infected colonies produce more and smaller males (Scharf et al. 2012). The infected ants however are more likely to be fertile upon queen removal (Beros et al. 2019), meaning their personal fitness can potentially increase thanks to parasite infection. Of course this thesis has shown on multiple occasions the profound impact the parasite still has on the host and ultimately the fitness of infected ants probably won't be higher as the fitness of social insects is mostly dependent on the productivity of the colony according to Hamilton's rule (Hamilton, 1964). However, in an infected colony it can be said that infected ants are at least in a far more advantageous position than their uninfected nestmates. With that, it is possible to pretend that infected ants are to uninfected workers what *A. brevis* is for infected ants. When we do this, we find surprising similarities. For example, we find various transport functions to be differential in both infected ants from colonies with a different parasite prevalence in **chapter 3** as well as cestodes from ants with a different parasite load in **chapter 1**. Though of course these transport functions would make much more sense in the cestode as they are endoparasites whereas we should count the infected ants as ectoparasites in this situation. Then, when regarding the "victim" of parasitism, or the uninfected ants in heavily infected colonies vs the infected ants with high *A. brevis* parasite load, I already mentioned the differential stress response.

Conclusion and future prospects

I believe the relevance of this thesis is two-fold. Firstly, through novel and unexpected methodologies, we have been able to identify multiple aspects of this complex parasite-host system. By investigating the parasite load we were able identify differences between parasite stress and parasite manipulation. Then, by adding another immune stressor, we could identify immune pathways both unique to the parasite but also identify the fact that our parasite is capable of modulating the host's phenotype, if only through a means of manipulating the immune response. Then finally, by introducing a completely new method of parasite-host gene expression analysis, we could see for the first time which genes are reactive to the host and which genes are manipulative to the host, as well as identify different completely unknown genes by their context in a gene expression network. In total this parasite-host interaction can be regarded as more complicated than we could have imagined four years ago when I started my project. But through this complexion shine answers that have not been answered yet for

many, more intensely studied parasite-host interactions. In this system that opens up many new ways to research this parasite-host interaction. Now that we have candidate cestode genes that are clearly involved with certain biological processes in the host we can start knocking down these genes to find out whether our predictions were correct regarding the interactome analysis in **chapter 3**. When receiving this double confirmation, it opens the door to many new avenues of parasitology in general, where figuring out the exact influence parasites have on their host has often been regarded as extremely difficult to precisely figure out (Reid & Berriman, 2013). Furthermore, I believe the methods used in **chapter 2** can prove to be extremely useful in figuring out specific immune pathways in a multitude of parasites and for the first time find out whether an immune response GO term in a non-model species is the result of parasite immune inhibition or a parasite induced immune response.

Finally, I would like to leave the thesis with some recommendations for future research, some of which are already partially implemented in our lab. For example, all the phenotypic changes caused by *A. brevis* to *T. nylanderi* start the moment the ant is infected, while the ant is still in its larval stage. Therefore, it is highly important to regard the developmental biology of *T. nylanderi* when infected with the parasite. In order to do this, it would be possible to find as many infected colonies as possible and immediately search for cestodes in larvae and callows, but the most precise solution would always be to artificially infect *T. nylanderi* with *A. brevis* us. During my PhD I've already done a few pilot experiments with this which were not successful and not big enough to be worthy of mentioning in this thesis, but different research efforts with other cestode systems have shown that it is possible to induce adult life stages in-vitro (Smyth, 1946; Wedekind et al. 1998; Weinreich et al. 2014). This might show that certain conclusions taken in this thesis are in fact wrong, especially regarding the development of the cuticle, since this part happens fully during ant development and there would be no need for the parasite to downregulate the cuticle sclerotization. Through artificial infections we can also start controlling for age in infected ants, which has always served as a difficult to circumvent side effect of infection. Just through simple statistics we can always assume that our infected ants are older than our uninfected ants, so the possibility could exist that all the gene expression differences we encounter are entirely age related and that the cestode might be a far more direct player through protein-protein interactions rather than gene expression manipulation, as low as this possibility might be upon a mountain of evidence that cestode infection is capable of altering host gene expression (Grecias et al. 2020; Morales-Montor & Larralde, 2005; Pierson et al. 2010). Furthermore, now that we understand some of these pathways that the cestode is capable of interfering with, we also need to understand how these pathways are being altered. For example, by looking at whether certain cestode proteins or regulatory RNA is capable of binding to certain host genes in order to silence them or interfere with their expression somehow. Whether the cestode might use epigenetic mechanisms to take control of the host. Developing these methods for this system specifically will need years of dedicated effort, but if there's something I've learned during my PhD, it's the resilience, durability and curiosity of a scientist can be endless, and throughout my PhD I've had the pleasure of meeting many of these interested in this parasite-host system, hopefully ready to answer these questions, which can help us understand some of the fundamental processes of life on earth.

References

Achour, Y. Ben, Chenik, M., Louzir, H., & Dellagi, K. (2002). Identification of a Disulfide Isomerase Protein of Leishmania major as a Putative Virulence Factor. *Fungal and Parasitic Infections*, *70*(7), 3576–3585. doi: 10.1128/IAI.70.7.3576

Ackerman, H., Usen, S., Jallow, M., Sisay-Joof, F., Pinder, M., & Kwiatkowski, D. P. (2005). A comparison of case-control and family-based association methods: The example of sickle-cell and malaria. *Annals of Human Genetics*, *69*(5), 559–565. doi: 10.1111/j.1529-8817.2005.00180.x

Ahmed-hassan, H., Sisson, B., Shukla, R. K., Wijewantha, Y., & Liyanage, N. P. M. (2020). *Innate Immune Responses to Highly Pathogenic Coronaviruses and Other Significant Respiratory Viral Infections*. *11*(August). doi: 10.3389/fimmu.2020.01979

Akhouayri, I., Turc, C., Royet, J., & Charroux, B. (2011). Toll-8/tollo negatively regulates antimicrobial response in the drosophila respiratory epithelium. *PLoS Pathogens*, *7*(10). doi: 10.1371/journal.ppat.1002319

Akino, T., Knapp, J. J., Thomas, J. A., & Elmes, G. W. (1999). Chemical mimicry and host specificity in the butterfly Maculinea rebeli, a social parasite of Myrmica ant colonies. *Proceedings of the Royal Society B: Biological Sciences*, *266*(1427), 1419–1426. doi: 10.1098/rspb.1999.0796

Al-Razi, A. B. (10th century). *The Comprehensive Book on Medicine*. Baghdad.

Alberro, A., Irabarren-Lopez, A., Sáenz-Cuesta, M., Matheu, A., Vergara, I., & Otaegui, D. (2021). Inflammaging markers characteristic of advanced age show similar levels with frailty and dependency. *Scientific Reports*, *11*(4358), 1–10. doi: 10.1038/s41598-021-83991-7

Albertyn, J., Hohmann, S., & Prior, B. A. (1994). Characterization of the osmotic-stress response in Saccharomyces cerevisiae: osmotic stress and glucose repression regulate glycerol-3-phosphate dehydrogenase independently. *Current Genetics*, *25*, 12–18.

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., & Lipman, D. J. (1990). Basic local alignment search tool. *Journal of Molecular Biology*, *215*(3), 403–410. doi: 10.1016/S0022-2836(05)80360-2

Alves, A. P. do C., Paulino, R. R., Pereira, R. T., da Costa, D. V., & e Rosa, P. V. (2021). Nile tilapia fed insect meal: Growth and innate immune response in different times under lipopolysaccharide challenge. *Aquaculture Research*, *52*(2), 529–540. doi: 10.1111/are.14911

Ambegaokar, S. S., Roy, B., & Jackson, G. R. (2010). Neurodegenerative models in Drosophila: Polyglutamine disorders, Parkinson disease, and amyotrophic lateral sclerosis. *Neurobiology of Disease*, *40*(1), 29–39. doi: 10.1016/j.nbd.2010.05.026

Amor, S., & Blanco, L. F. (2020). *Innate immunity during SARS-CoV-2 : evasion strategies and activation trigger hypoxia and vascular damage*. 193–209. doi: 10.1111/cei.13523

Ancarola, M. E., Lichtenstein, G., Herbig, J., Holroyd, N., Mariconti, M., Brunetti, E., … Cucher, M. (2020). Extracellular non-coding rna signatures of the metacestode stage of echinococcus multilocularis. *PLoS Neglected Tropical Diseases*, *14*(11), 1–27. doi: 10.1371/journal.pntd.0008890

Ancarola, M. E., Marcilla, A., Herz, M., Macchiaroli, N., Pérez, M., Asurmendi, S., … Cucher, M. (2017). Cestode parasites release extracellular vesicles with microRNAs and immunodiagnostic protein cargo. *International Journal for Parasitology*, *47*(10–11), 675–686. doi: 10.1016/j.ijpara.2017.05.003

Andersen, S. O. (2011). Cuticular Sclerotization and Tanning. In *Insect Molecular Biology and Biochemistry* (pp. 167–192). Elsevier B.V. doi: 10.1016/B978-0-12-384747-8.10006-6

Anderson, J. P., Gleason, C. A., Foley, R. C., Thrall, P. H., Burdon, J. B., & Singh, K. B. (2010). Plants versus pathogens : an evolutionary arms race. *Functional Plant Biology*, *37*, 499–512.

Andrews, S. (2010). *FastQC: A Quality Control Tool for High Throughput Sequence Data*. Retrieved from https://www.bioinformatics.babraham.ac.uk/projects/fastqc/

Arakane, Y., Lomakin, J., Beeman, R. W., Muthukrishnan, S., Gehrke, S. H., Kanost, M. R., & Kramer, K. J. (2009). Molecular and functional analyses of amino acid decarboxylases involved in cuticle tanning in Tribolium castaneum. *Journal of Biological Chemistry*, *284*(24), 16584–16594. doi: 10.1074/jbc.M901629200

Arrese, E. L., & Soulages, J. L. (2010). Insect fat body: Energy, metabolism, and regulation. *Annual Review of Entomology*, *55*, 207–225. doi: 10.1146/annurev-ento-112408-085356

Auld, S., & Tinsley, M. C. (2015). The evolutionary ecology of complex lifecycle parasites: linking phenomena with mechanisms. *Heridity*, *114*, 125–132. doi: 10.1038/hdy.2014.84

Avicenna. (11th century). *The Canon of Medicine (al-Qānūn fī al-Ṭibb)*.

Bach, J. F. (2018). The hygiene hypothesis in autoimmunity : the role of pathogens and commensals. *Nature Reviews Immunology*, *18*, 105–120. doi: 10.1038/nri.2017.111

Baid, G., Cook, D. E., Shafin, K., Yun, T., Llinares-López, F., Berthet, Q., … Carroll, A. (2023). DeepConsensus improves the accuracy of sequences with a gap-aware sequence transformer. *Nature Biotechnology*, *41*(February). doi: 10.1038/s41587-022-01435-7

Baker, A., Hogg, T. L., & Warriner, S. L. (2016). *Peroxisome protein import : a complex journey*. *0*, 783–789. doi: 10.1042/BST20160036

Baker, D. G. (2008). *Flynn's Parasites of Laboratory Animals*. Hoboken: Wiley.

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

Baker, P. F., & Knight, D. E. (1986). Exocytosis: control by calcium and other factors. *British Medical Bulletin*, *42*(4), 399–404.

Band, G., Leffler, E. M., Jallow, M., Sisay-Joof, F., Ndila, C. M., Macharia, A. W., … Kwiatkowski, D. P. (2022). Malaria protection due to sickle haemoglobin depends on parasite genotype. *Nature*, *602*(7895), 106–111. doi: 10.1038/s41586-021-04288-3

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. doi: 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.

Becker, K., Tilley, L., Vennerstrom, J. L., Roberts, D., Rogerson, S., & Ginsburg, H. (2004). Oxidative stress in malaria parasite-infected erythrocytes: Host-parasite interactions. *International Journal for Parasitology*, *34*(2), 163–189. doi: 10.1016/j.ijpara.2003.09.011

Behmer, S. T. (2009). Animal Behaviour : Feeding the Superorganism. *Current Biology*, *19*(9), R366–R368. doi: 10.1016/j.cub.2009.03.033

Benesh, D. P. (2019). Crowding in the first intermediate host does not affect infection probability in the second host in two helminths. *Journal of Helminthology*, *93*(2), 172–176. doi: 10.1017/S0022149X1800007X

Bennett, A., & Théry, M. (2020). Avian Color Vision and Coloration: Multidisciplinary Evolutionary Biology. *The American Naturalist*, *169*, 1–6.

Berger, C. S., Laroche, J., Maaroufi, H., Martin, H., Moon, K. M., Landry, C. R., … Aubin-Horth, N. (2021). The parasite Schistocephalus solidus secretes proteins with putative host manipulation functions. *Parasites and Vectors*, *14*(1). doi: 10.1186/s13071-021-04933-w

Beros, S., Enders, C., Menzel, F., & Foitzik, S. (2019). Parasitism and queen presence interactively shape worker behaviour and fertility in an ant host. *Animal Behaviour*, *148*, 63–70. doi: 10.1016/j.anbehav.2018.12.004

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). doi: 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). doi: 10.1098/rsos.202118

Bethel, W. M., & Holmes, J. C. (1973). Altered Evasive Behaviour and Responses to Light in Amphipods Harboring Acanthocephalan Cystacanths. *The Journal of Parasitology*, *59*(6), 945–956.

Bień, J., Sałamatin, R., Sulima, A., Savijoki, K., Conn, D. B., Näreaho, A., & Młocicki, D. (2016). Mass spectrometry analysis of the excretory-secretory (E-S) products of the model cestode Hymenolepis diminuta reveals their immunogenic properties and the presence of new E-S proteins in cestodes. *Acta Parasitologica*, *61*(2), 429–442. doi: 10.1515/ap-2016-0058

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). doi: 10.1016/J.ZOOL.2023.126119

Bisoffi, Z., Leoni, S., Angheben, A., Beltrame, A., Eseme, F. E., Gobbi, F., … Buonfrate, D. (2016). Chronic malaria and hyper-reactive malarial splenomegaly: a retrospective study on the largest series observed in a non-endemic country. *Malaria Journal*, *15*(230). doi: 10.1186/s12936-016-1274-x

Borges, D. C., Araújo, N. M., Cardoso, C. R., & Lazo Chica, J. E. (2012). Different parasite inocula determine the modulation of the immune response and outcome of experimental Trypanosoma cruzi infection. *Immunology*, *138*(2), 145–156. doi: 10.1111/imm.12022

Boulton, S. J., Brook, A., Staehling-Hampton, K., Heitzler, P., & Dyson, N. (2000). A role for Ebi in neuronal cell cycle control. *EMBO Journal*, *19*(20), 5376–5386. doi: 10.1093/emboj/19.20.5376

Briano, J., Calcaterra, L., & Varone, L. (2012). Fire ants (Solenopsis spp.) and their natural enemies in southern South America. *Psyche*, *2012*. doi: 10.1155/2012/198084

Brodbeck, D., Amherd, R., Callaerts, P., Hintermann, E., Meyer, U. A., & Affolter, M. (1998). Molecular and biochemical characterization of the aaNAT1 (Dat) locus in Drosophila melanogaster:

Differential expression of two gene products. *DNA and Cell Biology*, *17*(7), 621–633. doi: 10.1089/dna.1998.17.621

Broeck, J. Vanden. (2001). Insect G protein-coupled receptors and signal transduction. *Archives of Insect Biochemistry and Physiology*, *48*(1), 1–12. doi: 10.1002/arch.1054

Brown, S. P., Renaud, F., Gue, J., & Thomas, F. (2001). Evolution of trophic transmission in parasites : the need to reach a mating place ? *Journal of Evolutionary Biology*, *14*(5), 815–820. doi: 10.1046/j.1420-9101.2001.00318.x

Browne, N., Heelan, M., & Kavanagh, K. (2013). *An analysis of the structural and functional similarities of insect hemocytes and mammalian phagocytes*. 597–603.

Brumlik, M. J., Pandeswara, S., Ludwig, S. M., Jeansonne, D. P., Lacey, M. R., Murthy, K., … Curiel, T. J. (2013). TgMAPK1 is a Toxoplasma gondii MAP kinase that hijacks host MKK3 signals to regulate virulence and interferon-γ-mediated nitric oxide production. *Experimental Parasitology*, *134*(3), 389–399. doi: 10.1016/j.exppara.2013.03.016

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. doi: 10.1093/nargab/lqaa108

Buchfink, B., Xie, C., & Huson, D. H. (2014). Fast and sensitive protein alignment using DIAMOND. *Nature Methods*, *12*(1), 59–63. doi: 10.1038/nmeth.3176

Bush, A. O., Lotz, J. M., Bush, A. O., & Lotz, J. M. (2000). The ecology of “crowding.” *Journal of Parasitology*, *86*(2), 212–213. doi: 10.1645/0022-3395(2000)086[0212:TEOC]2.0.CO;2

Callahan, B. J., Mcmurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). *DADA2 : High-resolution sample inference from Illumina amplicon data*. *13*(7). doi: 10.1038/nmeth.3869

Calvani, E. N. D., De Marco Verissimo, C., Jewhurst, H. L., Cwiklinski, K., Flaus, A., & Dalton, J. P. (2022). Two Distinct Superoxidase Dismutases (SOD) Secreted by the Helminth Parasite Fasciola hepatica Play Roles in Defence against Metabolic and Host Immune Cell-Derived Reactive Oxygen Species (ROS) during Growth and Development. *Antioxidants*, *11*(10), 1–20.

Calvo, A. C., Pey, A. L., Ying, M., Loer, C. M., & Martinez, A. (2008). Anabolic function of phenylalanine hydroxylase in Caenorhabditis elegans. *The FASEB Journal*, *22*(8), 2601–3096. doi: 10.1096/fj.08-108522

Cancela, M., Paes, J. A., Moura, H., Barr, J. R., Zaha, A., & Ferreira, H. B. (2019). Unraveling oxidative stress response in the cestode parasite Echinococcus granulosus. *Scientific Reports*, *9*(1), 1–13. doi: 10.1038/s41598-019-52456-3

Cantalapiedra, C. P., Herṇandez-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. doi: 10.1093/molbev/msab293

Carthew, R. W. (2022). Gene Regulation and Cellular Metabolism: An Essential Partnership. *Trends in Genetics*, *37*(4), 389–400. doi: 10.1016/j.tig.2020.09.018.Gene

Casaravilla, C., Pittini, Á., Rückerl, D., Seoane, P. I., Jenkins, S. J., Macdonald, A. S., … 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. doi: 10.1128/IAI.01959-14

Catania, K. C. (2020). Getting the Most Out of Your Zombie: Abdominal Sensors and Neural Manipulations Help Jewel Wasps Find the Roach's Weak Spot. *Brain, Behaviour and Evolution*, *95*, 181–202. doi: 10.1159/000511548

Cézilly, F., Favrat, A., & Perrot-Minnot, M. J. (2013). Multidimensionality in parasite-induced phenotypic alterations: Ultimate versus proximate aspects. *Journal of Experimental Biology*, *216*(1), 27–35. doi: 10.1242/jeb.074005

Cézilly, F., Thomas, F., Médoc, V., & Perrot-Minnot, M. J. (2010). Host-manipulation by parasites with complex life cycles: adaptive or not? *Trends in Parasitology*, *26*(6), 311–317. doi: 10.1016/j.pt.2010.03.009

Chaplin, D. D. (2010). Overview of the Immune Response. *Journal of Allergy and Clinical Immunology*, *125*(2). doi: 10.1016/j.jaci.2009.12.980.Overview

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. doi: 10.1098/rspb.2007.0531

Charles, H. M., & Killian, K. A. (2015). Response of the insect immune system to three different immune challenges. *Journal of Insect Physiology*, *81*, 97–108. doi: 10.1016/j.jinsphys.2015.07.005

Chehayeb, J. F., Robertson, A. P., Martin, R. J., & Geary, T. G. (2014). Proteomic Analysis of Adult Ascaris suum Fluid Compartments and Secretory Products. *PLoS Neglected Tropical Diseases*, *8*(6). doi: 10.1371/journal.pntd.0002939

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

Choe, K. M., Werner, T., Stöven, S., Hultmark, D., & Anderson, K. V. (2002). Requirement for a peptidoglycan recognition protein (PGRP) in relish activation and antibacterial immune responses in Drosophila. *Science*, *296*(5566), 359–362. doi: 10.1126/science.1070216

Chora, F., Mota, M. M., & Mukherjee, D. (2020). *Microbiota , a Third Player in the Host – Plasmodium Affair*. *36*(1), 11–18. doi: 10.1016/j.pt.2019.11.001

Chulanetra, M., & Chaicumpa, W. (2021). *Revisiting the Mechanisms of Immune Evasion Employed by Human Parasites*. *11*(July), 1–32. doi: 10.3389/fcimb.2021.702125

Clarke, A. S. (1954). Studies on the life cycle of the pseudophyllidean cestode Schistocephalus solidus. *Proceedings of the Zoological Society of London*, *124*(2), 257–302. doi: 10.1111/j.1469-7998.1954.tb07782.x

Clayton, N. S., Mellor, R., & Jackson, A. (1996). Seasonal patterns of food storing in the Jay Garrulus glandarius. *Ibis*, *138*(2), 250–255. doi: 10.1111/j.1474-919x.1996.tb04336.x

Coakley, G., Buck, A. H., & Maizels, R. M. (2016). Host parasite communications—Messages from helminths for the immune system: Parasite communication and cell-cell interactions. *Molecular and Biochemical Parasitology*, *208*(1), 33–40. doi: 10.1016/j.molbiopara.2016.06.003

Coakley, G., Maizels, R. M., & Buck, A. H. (2015). Exosomes and Other Extracellular Vesicles: The New

Communicators in Parasite Infections. *Trends in Parasitology*, *31*(10), 477–489. doi: 10.1016/j.pt.2015.06.009

Colella, P., Sellier, P., Gomez, M. J., Biferi, M. G., Tanniou, G., Guerchet, N., … Mingozzi, F. (2020). Gene therapy with secreted acid alpha-glucosidase rescues Pompe disease in a novel mouse model with early-onset spinal cord and respiratory defects. *EBioMedicine*, *61*. doi: 10.1016/j.ebiom.2020.103052

Colinet, D., Schmitz, A., Cazes, D., Gatti, J. L., & Poirié, M. (2010). The origin of intraspecific variation of virulence in an eukaryotic immune suppressive parasite. *PLoS Pathogens*, *6*(11). doi: 10.1371/journal.ppat.1001206

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. doi: 10.1016/S0092-8674(03)00713-X

Coon, K. L., Vogel, K. L., Brown, M. R., & Strand, M. R. (2014). Mosquitoes rely on their gut microbiota for development. *Molecular Ecology*, *23*(11), 2727–2739. doi: 10.1111/mec.12771

Cornett, S., & Sorci, G. (2010). Parasite virulence when the infection reduces the host immune response. *Proceedings of the Royal Society B: Biological Sciences*, 1929–1935. doi: 10.1098/rspb.2010.0138

Cost, G. J., Freyvert, Y., Vafiadis, A., Santiago, Y., Miller, J. C., Rebar, E., … Boulevard, C. (2010). BAK and BAX Deletion Using Zinc-Finger Nucleases Yields Apoptosis-Resistant CHO Cells. *Biotechnology and Bioengineering*, *105*(2), 330–340. doi: 10.1002/bit.22541

Crawley, M. J. (1997). *Plant ecology*. Oxford: Blackwell Scientific Publications.

Cremer, S., Armitage, S. A. O., & Schmid-Hempel, P. (2007). Social Immunity. *Current Biology*, *17*(16), 693–702. doi: 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. doi: 10.1146/annurev-ento-020117-043110

Cribb, T. H., Bray, R. A., Olson, P. D., & Littlewood, D. T. J. (2003). Life Cycle Evolution in the Digenea : a New Perspective from Phylogeny. *Advances in Parasitology*, *54*, 198–254. doi: https://doi.org/10.1016/S0065-308X(03)54004-0

Critchlow, J. T., Norris, A., Tate, A. T., & Tate, A. T. (2019). The legacy of larval infection on immunological dynamics over metamorphosis. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *374*(20190066).

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. doi: 10.1890/070151

Crump, G. M., Mashayekh, S., Zhou, J., & Grimes, C. L. (2021). *HHS Public Access*. *56*(87), 13313–13322. doi: 10.1039/d0cc02605k.Revisiting

Dass, S. A. H., & Vyas, A. (2014). Toxoplasma gondii infection reduces predator aversion in rats through epigenetic modulation in the host medial amygdala. *Molecular Ecology*, *23*(24), 6114–6122. doi: 10.1111/mec.12888

Davis, F. P., Barkan, D. T., Eswar, N., Mckerrow, J. H., & Sali, A. (2009). Host – pathogen protein interactions predicted by comparative modeling. *Protein Science*. doi: 10.1110/ps.073228407.6

Davis, N. M., Proctor, Di. M., Holmes, S. P., Relman, D. A., & Callahan, B. J. (2018). Simple statistical identification and removal of contaminant sequences in marker-gene and metagenomics data. *Microbiome*, *6*(1), 1–14. doi: 10.1186/s40168-018-0605-2

Davla, S., Artiushin, G., Li, Y., Chitsaz, D., Li, S., Sehgal, A., & van Meyel, D. J. (2020). AANAT1 functions in astrocytes to regulate sleep homeostasis. *ELife*, *9*(e53994), 1–18. doi: 10.7554/ELIFE.53994

Dawkins, R. (1982). *The Extended Phenotype*. Oxford: Oxford University Press.

De Coster, G., De Neve, L., Verhulst, S., & Lens, L. (2012). Maternal effects reduce oxidative stress in female nestlings under high parasite load. *Journal of Avian Biology*, *43*(2), 177–185. doi: 10.1111/j.1600-048X.2012.05551.x

De Roode, J. C., Gold, L. R., & Altizer, S. (2007). Virulence determinants in a natural butterfly-parasite system. *Parasitology*, *134*(5), 657–668. doi: 10.1017/S0031182006002009

De Roode, Jacobus C., Yates, A. J., & Altizer, S. (2008). Virulence-transmission trade-offs and population divergence in virulence in a naturally occurring butterfly parasite. *Proceedings of the National Academy of Sciences of the United States of America*, *105*(21), 7489–7494. doi: 10.1073/pnas.0710909105

De Roode, Jacobus C, Helinski, M. E. H., Anwar, M. A., & Read, A. F. (2005). Dynamics of Multiple Infection and Within-Host Competition in Genetically Diverse Malaria Infections. *The American Naturalist*, *166*(5), 531. doi: 10.2307/3491213

Dence, W. A. (1958). Studies on Ligula-Infected Common Shiners (Notropis cornutus frontalis Agassiz) in the Adirondacks. *The Journal of Parasitology*, *44*(3), 334–338.

Dermauw, W., Van Leeuwen, T., & Feyereisen, R. (2020). Diversity and evolution of the P450 family in arthropods. *Insect Biochemistry and Molecular Biology*, *127*. doi: 10.1016/j.ibmb.2020.103490

Diangelo, J. R., Bland, M. L., Bambina, S., Cherry, S., & Birnbaum, M. J. (2009). The immune response attenuates growth and nutrient storage in Drosophila by reducing insulin signaling. *Proceedings of the National Academy of Sciences of the United States of America*, *106*(49), 20853–20858. doi: 10.1073/pnas.0906749106

Dimarcq, J., Hoffmann, D., Meister, M., Bulet, P., Lanot, R., Reichhart, J., & Hoffmann, J. A. (1994). Characterization and transcriptional profiles of a Drosophila gene encoding an insect defensin. *European Journal of Biochemistry*, *221*(1), 201–209.

Dingemanse, N. J., Oosterhof, C., Van Der Plas, F., & Barber, I. (2009). Variation in stickleback head morphology associated with parasite infection. *Biological Journal of the Linnean Society*, *96*(4), 759–768. doi: 10.1111/j.1095-8312.2008.01179.x

Dobell, C. (1932). *Antony van Leeuwenhoek and his "little animals."* London: John Bale Sons and Danielsson.

Dobin, A., Davis, C. A., Schlesinger, F., Drenkow, J., Zaleski, C., Jha, S., … Gingeras, T. R. (2013). *Sequence analysis*. *29*(1), 15–21. doi: 10.1093/bioinformatics/bts635

Dow, J. A. T. (2017). The essential roles of metal ions in insect homeostasis and physiology. *Current Opinion in Insect Science*, *23*, 43–50. doi: 10.1016/j.cois.2017.07.001

Dumont, M., Moné, H., Mouahid, G., Idris, M. A., Shaban, M., & Boissier, J. (2007). Influence of pattern of exposure, parasite genetic diversity and sex on the degree of protection against reinfection with Schistosoma mansoni. *Parasitology Research*, *101*(2), 247–252. doi: 10.1007/s00436-007-0476-0

Dunn, A. M., Torchin, M. E., Hatcher, M. J., Kotanen, P. M., & Dana, M. (2012). Indirect effects of parasites in invasions. *Functional Ecology*, *26*(6), 1262–1274. doi: 10.1111/j.1365-2435.2012.02041.x

Duplais, C., Sarou-kanian, V., Massiot, D., Hassan, A., Perrone, B., Estevez, Y., … Moreau, C. S. (2021). Gut bacteria are essential for normal cuticle development in herbivorous turtle ants. *Nature Communications*, *12*(676). doi: 10.1038/s41467-021-21065-y

Dyer, M. D., Murali, T. M., & Sobral, B. W. (2007). Computational prediction of host-pathogen protein – protein interactions. *Bioinformatics*. doi: 10.1093/bioinformatics/btm208

Dzik, J. M. (2006). Molecules released by helminth parasites involved in host colonization Molecules released by helminth parasites involved in host colonization. *Acta Biochimica Polonica*, *53*(1), 33–64. doi: 10.18388/abp.2006

Ebbell, B. (1937). *The Papyrus Ebers: the greatest Egyptian medical document*. Copenhagen: Levin & Minksgaard.

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. doi: 10.3389/fimmu.2018.01868

El-Beshbishi, S. N., Ahmed, N. N., Mostafa, S. H., & El-Ganainy, G. A. (2012). Parasitic infections and myositis. *Parasitology Research*, *110*(1), 1–18. doi: 10.1007/s00436-011-2609-8

Elliott, D. E., & Weinstock, J. V. (2012). Helminth-host immunological interactions: Prevention and control of immune-mediated diseases. *Annals of the New York Academy of Sciences*, *1247*(1), 83–96. doi: 10.1111/j.1749-6632.2011.06292.x

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

Evans, H. C., Elliot, S. L., & Hughes, D. P. (2011). *Ophiocordyceps unilateralis*. *4*(5), 598–602. doi: 0.1371/journal.pone

Ezema, C. A., Uzochukwu, I., Timothy, O., & Chidike, P. (2023). Escaping the enemy ' s bullets : an update on how malaria parasites evade host immune response. *Parasitology Research*, 1715–1731. doi: 10.1007/s00436-023-07868-6

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. doi: 10.1111/mec.13498

Ferguson, S. T., Park, K. Y., Ruff, A. A., Bakis, I., & Zwiebel, L. J. (2020). Odor coding of nestmate recognition in the eusocial ant Camponotus floridanus. *Journal of Experimental Biology*, *223*(2). doi: 10.1242/jeb.215400

Ferreira, A. V, Koeken, V. A. C. M., Matzaraki, V., Kostidis, S., Alarcon-barrera, J. C., Bree, L. C. J. De, …

Dom, J. (2021). *Glutathione Metabolism Contributes to the Induction of Trained Immunity*. 1–11.

Finkel, T., & Holbrook, N. J. (2000). Oxidants, oxidative stress and the biology of ageing. *Nature*, *408*(November), 239–247.

Flegr, J., Hlavá, J., & Toman, J. (2023). Parasitic manipulation or side effects? The effects of past Toxoplasma and Borrelia infections on human personality and cognitive performance are not mediated by impaired health. *MedRxiv*. doi: 10.1101/2023.01.07.23284298

Folick, A., Oakley, H. D., Yu, Y., Armstrong, E. H., Kumari, M., Sanor, L., … Wang, M. C. (2015). Lysosomal signaling molecules regulate longevity in Caenorhabditis elegans. *Science*, *347*(6217), 83–86. doi: 10.1126/science.1258857

Forrester, G. E., & Finley, R. J. (2006). Parasitism and a shortage of refuges jointly mediate the strength of density dependence in a reef fish. *Ecology*, *87*(5), 1110–1115.

Fortini, M. E., & Bonini, N. M. (2000). Modeling human neurodegenerative diseases in Drosophila: On a wing and a prayer. *Trends in Genetics*, *16*(4), 161–167. doi: 10.1016/S0168-9525(99)01939-3

Foster, G. W., Kinsella, J. M., Walters, E. L., Schrader, M. S., & Forrester, D. J. (2002). Parasitic helminths of red-bellied woodpeckers (Melanerpes carolinus) from the Apalachicola National Forest in Florida. *Journal of Parasitology*, *88*(6), 1140–1142. doi: 10.2307/3285485

Franceschi, N., Bauer, A., Bollache, L., & Rigaud, T. (2008). The effects of parasite age and intensity on variability in acanthocephalan-induced behavioural manipulation. *International Journal for Parasitology*, *38*(10), 1161–1170. doi: 10.1016/j.ijpara.2008.01.003

Frank, S. A. (1996). Models of parasite virulence. *The Quarterly Review of Biology*, *71*(1), 37–78.

Fredensborg, B. L., Fossdal Í Kálvalíð, I., Johannesen, T. B., Stensvold, C. R., Nielsen, H. V., & Kapel, C. M. O. (2020). Parasites modulate the gut-microbiome in insects: A proof-of-concept study. *PloS One*, *15*(1), e0227561. doi: 10.1371/journal.pone.0227561

Fuchs, S., Behrends, V., Bundy, J. G., Crisanti, A., & Nolan, T. (2014). Phenylalanine Metabolism Regulates Reproduction and Parasite Melanization in the Malaria Mosquito. *PLoS ONE*, *9*(1). doi: 10.1371/journal.pone.0084865

Fuhrmann, O. (1909). *Die Cestoden der Vögel*. Leopold Classic Library.

Gabrion, C., & Helluy, S. (1982). Développement larvaire de Paricterotaenia porosa (Cestoda: Cyclophyllidea, Dilepididae) chez des Diptères du genre Chironomus, hôtes expérimentaux. *Annales de Parasitologie Humaine et Comparée*, *57*(1), 33–52. doi: 10.1051/parasite/1982571033

Gamper, N., & Shapiro, M. S. (2007). Regulation of ion transport proteins by membrane phosphoinositides. *Nature Reviews Neuroscience*, *8*(12), 921–934. doi: 10.1038/nrn2257

Gatkowska, J., Wieczorek, M., Dziadek, B., Dzitko, K., & Dlugonska, H. (2012). Behavioral changes in mice caused by Toxoplasma gondii invasion of brain. *Parasitology Research*, *111*(1), 53–58. doi: 10.1007/s00436-011-2800-y

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. doi: 10.1007/s12639-012-0229-6

Gillespie, J. P., Kanost, M. R., & Trenczek, T. (1997). Biological mediators of insect immunity. *Annual*

Review of Entomology, *42*, 661–643.

Good, M. F., Xu, H., Wykes, M., & Engwerda, C. R. (2005). Development and Regulation of Cell-mediated Immune Responses to the Blood Stages of Malaria: Implications for Vaccine Research. *Annual Review of Immunology*, *23*, 69–99. doi: 10.1146/annurev.immunol.23.021704.115638

Gorman, G. (2015). Foraging signs and cavities of some European woodpeckers (Picidae): Identifying the clues that lead to establishing the presence of species. *Denisia*, *36*(164), 87–97.

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). doi: 10.1371/journal.pone.0007713

Grabowicz, M., Andres, D., Lebar, M. D., Maloj, G., Kahne, D., & Silhavy, T. J. (2014). *A mutant Escherichia coli that attaches peptidoglycan to lipopolysaccharide and displays cell wall on its surface*. 1–11. doi: 10.7554/eLife.05334

Grecias, L., Hebert, F. O., Alves, V. A., Barber, I., Aubin-horth, N., & Aubin-horth, N. (2020). Host behaviour alteration by its parasite : from brain gene expression to functional test. *Proceedings of the Royal Society B: Biological Sciences*, *20202252*.

Gruber, J., Ng, L. F., Fong, S., Wong, Y. T., Koh, S. A., Chen, C., … Halliwell, B. (2011). Mitochondrial Changes in Ageing Caenorhabditis elegans – What Do We Learn from Superoxide Dismutase Knockouts? *PLoS ONE*, *6*(5). doi: 10.1371/journal.pone.0019444

Ha, E. M., Oh, C. T., Bae, Y. S., & Lee, W. J. (2005). A direct role for dual oxidase in Drosophila gut immunity. *Science*, *310*(5749), 847–850. doi: 10.1126/science.1117311

Haas, B. J., Papanicolaou, A., Yassour, M., Grabherr, M., Blood, P. D., Bowden, J., … Regev, A. (2013). De novo transcript sequence reconstruction from RNA-seq using the Trinity platform for reference generation and analysis. *Nature Protocols*, *8*(8), 1494–1512. doi: 10.1038/nprot.2013.084

Habash, S. S., Miroslaw, S., Siddique, S., Voigt, B., Elashry, A., & Grundler, F. W. M. (2017). Identification and characterization of a putative protein disulfide isomerase (HsPDI) as an alleged effector of Heterodera schachtii. *Scientific Reports*, *7*. doi: 10.1038/s41598-017-13418-9

Hahn, M. A., Piecyk, A., Jorge, F., Cerrato, R., Kalbe, M., & Dheilly, N. M. (2022). Host phenotype and microbiome vary with infection status, parasite genotype, and parasite microbiome composition. *Molecular Ecology*, *31*, 1577–1594. doi: 10.1111/mec.16344

Halliez, M. C. M., & Buret, A. G. (2015). Gastrointestinal Parasites and the Neural Control of Gut Functions. *Frontiers in Cellular Neuroscience*, *9*(452). doi: 10.3389/fncel.2015.00452

Halliwell, B., & Gutteridge, J. M. C. (1989). *Free Radicals in Biology and Medicine*. Oxford: Oxford University Press.

Hamilton, W. D. (1964). The genetical evolution of social behavior. I. *Journal of Theoretical Biology*, 23–43. doi: 10.4324/9780203790427-4

Hamilton, W. D. (1970). Selfish and spiteful behaviour in an evolutionary model. *Nature*, *228*, 1218–1220.

Hammerschmidt, K., & Kurtz, J. (2005). Evolutionary implications of the adaptation to different immune systems in a parasite with a complex life cycle. *Proceedings of the Royal Society B: Biological Sciences*, *272*, 2511–2518. doi: 10.1098/rspb.2005.3241

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 brasiliensis and the evolution of secreted. *Genome Biology*, *5*(6).

Harman, D. (2002). Aging: a theory based on free radical and radiation chemistry. *Science of Aging Knowledge Environment*, *2002*(37), 289–291.

Harris, E, Deacon, A. C., Bray, R., & Hoodless, A. N. (2018). Is cestode infection intensity associated with decreased body condition in the Eurasian woodcock Scolopax rusticola? *Journal of Helminthology*, *92*(1), 42–48. doi: 10.1017/S0022149X17000037

Harris, G., Shen, Y., Ha, H., Donato, A., Wallis, S., Zhang, X., & Zhang, Y. (2014). Dissecting the signaling mechanisms underlying recognition and preference of food odors. *Journal of Neuroscience*, *34*(28), 9389–9403. doi: 10.1523/JNEUROSCI.0012-14.2014

Harris, Erica V, Roode, J. C. De, & Id, N. M. G. (2019). *Diet – microbiome – disease : Investigating diet ' s influence on infectious disease resistance through alteration of the gut microbiome*. (Fig 1). doi: 10.1371/journal.ppat.1007891

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). doi: 10.1098/rstb.2017.0205

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

Hartley, A. M., Lukoyanova, N., Zhang, Y., Cabrera-Orefice, A., Arnold, S., Meunier, B., … Maréchal, A. (2019). Structure of yeast cytochrome c oxidase in a supercomplex with cytochrome bc 1. *Nature Structural and Molecular Biology*, *26*(1), 78–83. doi: 10.1038/s41594-018-0172-z

Hartmann, W., Haben, I., Fleischer, B., & Breloer, M. (2011). Pathogenic Nematodes Suppress Humoral Responses to Third-Party Antigens In Vivo by IL-10–Mediated Interference with Th Cell Function . *Journal of Immunology*, *187*(8), 4088–4099. doi: 10.4049/jimmunol.1004136

Hayes, J. D., Flanagan, J. U., & Jowsey, I. R. (2005). Glutathione transferases. *Annual Review of Pharmacology and Toxicology*, *45*, 51–88. doi: 10.1146/annurev.pharmtox.45.120403.095857

Hayman, D. T. S., Emmerich, P., Yu, M., Wang, L. F., Suu-Ire, R., Fooks, A. R., … Wood, J. L. N. (2010). Long-term survival of an urban fruit bat seropositive for ebola and lagos bat viruses. *PLoS ONE*, *5*(8). doi: 10.1371/journal.pone.0011978

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. doi: 10.1186/s12859-015-0611-3

Heil, M. (2016). Host manipulation by parasites: Cases, patterns, and remaining doubts. *Frontiers in Ecology and Evolution*, *4*(80). doi: 10.3389/fevo.2016.00080

Heins, D. C., Baker, J. A., & Martin, H. C. (2002). The "Crowding Effect" in the Cestode Schistocephalus solidus : Density-Dependent Effects on Plerocercoid Size and Infectivity. *The Journal of Parasitology*, *88*(2), 302–307.

Helantera, H. (2021). Pathways to parasitic strategies in ants. *Proceedings of the National Academy of*

Sciences of the United States of America, *118*(41), 10–12. doi: 10.1073/pnas.2115607118

Helmby, H. (2015). Human helminth therapy to treat inflammatory disorders- where do we stand ? *BMC Immunology*, *16*(12), 1–5. doi: 10.1186/s12865-015-0074-3

Hernandez, J. L. R., Leung, G., & McKay, D. M. (2013). Cestode regulation of inflammation and inflammatory diseases. *International Journal for Parasitology*, *43*(3–4), 233–243. doi: 10.1016/j.ijpara.2012.09.005

Hicks, O., Burthe, S. J., Daunt, F., Newell, M., Chastel, O., Parenteau, C., & Green, J. A. (2018). The role of parasitism in the energy management of a free-ranging bird. *Journal of Experim*, *221*(24). doi: 10.1242/jeb.190066

Hill, A. V. S., Allsopp, C. E. M., Kwiatkowski, D., Anstey, N. M., Twumasi, P., Rowe, P. A., … Greenwood, B. M. (1991). Common West African HLA antigens are associated with protection from severe malaria. *Nature*, *352*(6336), 595–600. doi: 10.1038/352595a0

Hirota, Y., Sawamoto, K., Takahashi, K., Ueda, R., & Okano, H. (2005). The transmembrane protein, Tincar, is involved in the development of the compound eye in Drosophila melanogaster. *Development Genes and Evolution*, *215*(2), 90–96. doi: 10.1007/s00427-004-0452-y

Hoffmann, J. A. (1995). *Innate immunity of insects*. 4–10.

Hoffmann, J. A., & Reichhart, J. (2002). Drosophila innate immunity: an evolutionary perspective. *Nature Immunology*, *3*(2), 121–126.

Holmes, J. (1961). Effects of concurrent infections on Hymenolepis diminuta (cestoda) and Moniliformis dubius (acanthocephala). I. General effects and Comparison with crowding. *The Journal of Parasitology*, *47*(2), 209–216. doi: 10.1645/0022-3395(2002)088[0434:EOCIOH]2.0.CO;2

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

Hurd, H., Warr, E., & Polwart, A. (2001). A parasite that increases host lifespan. *Proceedings of the Royal Society B: Biological Sciences*, *268*(1477), 1749–1753. doi: 10.1098/rspb.2001.1729

Hurford, A., & Day, T. (2013). Immune Evasion And The Evolution Of Molecular Mimicry In Parasites. *Evolution*, *67*(10), 2889–2904. doi: 10.1111/evo.12171

Hwangbo, D.-S., Lee, H.-Y., Abozaid, L. S., & Min, K.-J. (2020). Mechanisms of Lifespan Regulation by Calorie Restriction and Intermittent Fasting in Model Organisms. *Nutrients*, *12*(1194).

Id, K. R. E., & Id, W. E. G. (2020). *Manipulating neutrophil degranulation as a bacterial virulence strategy*. 1–7. doi: 10.1371/journal.ppat.1009054

Imler, J., Tauszig, S., Jouanguy, E., Forestier, C., & Hoffmann, J. A. (n.d.). *LPS-induced immune response in Drosophila*.

Inan, M., Aryasomayajula, D., Sinha, J., & Meagher, M. M. (2006). Enhancement of Protein Secretion in Pichia pastoris by Overexpression of Protein Disulfide Isomerase. *Biotechnology and*

Bioengineering, *93*(4), 771–778. doi: 10.1002/bit.20762

Ingram, W. M., Goodrich, L. M., Robey, E. A., & Eisen, M. B. (2013). Mice Infected with Low-Virulence Strains of Toxoplasma gondii Lose Their Innate Aversion to Cat Urine, Even after Extensive Parasite Clearance. *PLOS ONE*, *8*(9), e75246. Retrieved from https://doi.org/10.1371/journal.pone.0075246

Izvekova, G. I., & Frolova, T. V. (2017). Proteolytic enzymes and their inhibitors in cestodes. *Biology Bulletin Reviews*, *7*(2), 150–159. doi: 10.1134/s2079086417020049

Izvekova, Galina I., Frolova, T. V., Izvekov, E. I., Kashinskaya, E. N., & Solovyev, M. M. (2021). Localization of the proteinase inhibitor activity in the fish cestode Eubothrium rugosum. *Journal of Fish Diseases*, *44*(12), 1951–1958. doi: 10.1111/jfd.13508

Jasti, A., Ojha, S. C., & Singh, Y. I. (2007). Mental and behavioral effects of parasitic infections: a review. *Nepal Medical College Journal*, *9*(1), 50–56.

Ji, C., Shen, H., Su, C., Li, Y., Chen, S., Sharp, T. H., & Xiao, J. (2023). Plasmodium falciparum has evolved multiple mechanisms to hijack human immunoglobulin M. *Nature Communications*, *14*(2650). doi: 10.1038/s41467-023-38320-z

Jiang, H., Lkhagva, A., Daubnerová, I., Chae, H. S., Šimo, L., Jung, S. H., … Kim, Y. J. (2013). Natalisin, a tachykinin-like signaling system, regulates sexual activity and fecundity in insects. *Proceedings of the National Academy of Sciences of the United States of America*, *110*(37). doi: 10.1073/pnas.1310676110

Johannessen, O. H. (1973). Deformations of the inner shell surface of venerupis pull astra (Montagu) (Lamellibranchia) as a result of infection by a trematcd metacercaria: With a note of parasitism leading to parasitic castration. *Sarsia*, *52*(1), 117–122. doi: 10.1080/00364827.1973.10411235

Johnson, C. A., Topoff, H., Vander Meer, R. K., & Lavine, B. (2005). Do these eggs smell funny to you?: An experimental study of egg discrimination by hosts of the social parasite Polyergus breviceps (Hymenoptera: Formicidae). *Behavioral Ecology and Sociobiology*, *57*(3), 245–255. doi: 10.1007/s00265-004-0851-0

Johnson, P. T. J., & Sutherland, D. R. (2003). Amphibian deformities and Ribeiroia infection: An emerging helminthiasis. *Trends in Parasitology*, *19*(8), 332–335. doi: 10.1016/S1471-4922(03)00148-X

Johnston, P. R., & Rolff, J. (2013). Immune- and wound-dependent differential gene expression in an ancient insect. *Developmental and Comparative Immunology*, *40*(3–4), 320–324. doi: 10.1016/j.dci.2013.01.012

Jojić, V., Čabrilo, B., Čabrilo, O. B., Jovanović, V. M., & Budinski, I. (2021). Canalization and developmental stability of the yellow - necked mouse (Apodemus flavicollis) mandible and cranium related to age and nematode parasitism. *Frontiers in Zoology*, *18*(55). doi: 10.1186/s12983-021-00439-4

Jones, P., Binns, D., Chang, H. Y., Fraser, M., Li, W., McAnulla, C., … Hunter, S. (2014). InterProScan 5: Genome-scale protein function classification. *Bioinformatics*, *30*(9), 1236–1240. doi: 10.1093/bioinformatics/btu031

Jong, M. D. De, Simmons, C. P., Thanh, T. T., Hien, V. M., Smith, G. J. D., Nguyen, T., … Farrar, J. (2006). Fatal outcome of human influenza A (H5N1) is associated with high viral load and

hypercytokinemia. *Nature Medicine*, *12*(10), 1203–1207. doi: 10.1038/nm1477

Jongepier, E., Séguret, A., Labutin, A., Feldmeyer, B., Gstöttl, C., Foitzik, S., … Bornberg-Bauer, E. (2022). Convergent Loss of Chemoreceptors across Independent Origins of Slave-Making in Ants. *Molecular Biology and Evolution*, *39*(1). doi: 10.1093/molbev/msab305

Kaltungo, B. Y., & Musa, I. W. (2013). A Review of Some Protozoan Parasites Causing Infertility in Farm Animals. *ISRN Tropical Medicine*. doi: 10.1155/2013/782609

Kan, H., Kim, C.-H., Kwon, H.-M., Park, J.-W., Roh, K.-B., Lee, H., … Lee, B. L. (2008). Molecular Control of Phenoloxidase-induced Melanin Synthesis in an Insect. *The Journal of Biological Chemistry*, *283*(37), 25316–25323.

Karsai, I., & Wenzel, J. W. (1998). Productivity, individual-level and colony-level flexibility, and organization of work as consequences of colony size. *Proceedings of the National Academy of Sciences of the United States of America*, *95*(15), 8665–8669. doi: 10.1073/pnas.95.15.8665

Kashiide, T., Kikuta, S., Yamaguchi, M., Irie, T., Kouguchi, H., Yagi, K., & Matsumoto, J. (2018). Molecular and functional characterization of glucose transporter genes of the fox tapeworm Echinococcus multilocularis. *Molecular and Biochemical Parasitology*, *225*(August), 7–14. doi: 10.1016/j.molbiopara.2018.08.004

Kaunisto, K. M., & Suhonen, J. (2013). Parasite burden and the insect immune response: Interpopulation comparison. *Parasitology*, *140*(1), 87–94. doi: 10.1017/S0031182012001369

Keane, R. M., & Crawley, M. J. (2002). Exotic plant invasions and the enemy release hypothesis. *Trends in Ecology and Evolution*, *17*(4), 164–170. doi: 10.1016/S0169-5347(02)02499-0

Khoury, L. Y. E., Campbell, J. M., & Clark, K. J. (2018). The transition of zebrafish functional genetics from random mutagenesis to targeted integration. In *Molecular-Genetic and Statistical Techniques for Behavioral and Neural Research*. Elsevier Inc. doi: 10.1016/B978-0-12-804078-2.00017-9

Kikawada, T., Saito, A., Kanamori, Y., Nakahara, Y., Iwata, K. I., Tanaka, D., … 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 of the United States of America*, *104*(28), 11585–11590. doi: 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. doi: 10.1038/nmeth.3317

Kim, M. S., Baek, M. J., Lee, M. H., Park, J. W., Lee, S. Y., Söderhäll, K., & Lee, B. L. (2002). A new easter-type serine protease cleaves a masquerade-like protein during prophenoloxidase activation in Holotrichia diomphalia larvae. *Journal of Biological Chemistry*, *277*(42), 39999–40004. doi: 10.1074/jbc.M205508200

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

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

Klammsteiner, T., Walter, A., Bogataj, T., Heussler, C. D., Stres, B., Steiner, F. M., … Jordan, H. R.

(2020). The Core Gut Microbiome of Black Soldier Fly (Hermetia illucens) Larvae Raised on Low-Bioburden Diets. *Frontiers in Microbiology*, *11*, 1–14. doi: 10.3389/fmicb.2020.00993

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. doi: 10.1038/s41587-019-0072-8

Kondo, Y., Yoda, S., Mizoguchi, T., Ando, T., Yamaguchi, J., & Yamamoto, K. (2017). Toll ligand Spätzle3 controls melanization in the stripe pattern formation in caterpillars. *Proceedings of the National Academy of Sciences of the United States of America*, *114*(31), 8336–8341. doi: 10.1073/pnas.1707896114

Konrad, M., Vyleta, M. L., Theis, F. J., Stock, M., Tragust, S., Klatt, M., … Cremer, S. (2012). Social Transfer of Pathogenic Fungus Promotes Active Immunisation in Ant Colonies. *PLoS Biology*, *10*(4). doi: 10.1371/journal.pbio.1001300

Korner, P., & Schmid-Hempel, P. (2004). In vivo dynamics of an immune response in the bumble bee Bombus terrestris. *Journal of Invertebrate Pathology*, *87*(1), 59–66. doi: 10.1016/j.jip.2004.07.004

Kramer, B. H., Nehring, V., Buttstedt, A., Heinze, J., Korb, J., Libbrecht, R., … Bernadou, A. (2021). Oxidative stress and senescence in social insects: A significant but inconsistent link? *Philosophical Transactions of the Royal Society B: Biological Sciences*, *376*(1823). doi: 10.1098/rstb.2019.0732

Krams, I. A., Kecko, S., Jõers, P., Trakimas, G., Elferts, D., Krams, R., … Krama, T. (2017). Microbiome symbionts and diet diversity incur costs on the immune system of insect larvae. *Journal of Experimental Biology*, *220*, 4204–4212. doi: 10.1242/jeb.169227

Krasikov, V. V, Karelov, D. V, & Firsov, L. M. (2001). α-Glucosidases. *Biochemistry*, *66*(3).

Krautz, R., Arefin, B., & Theopold, U. (2014). Damage signals in the insect immune response. *Frontiers in Plant Science*, *5*(JUL), 1–11. doi: 10.3389/fpls.2014.00342

Kremer, K., Kamin, D., Rittweger, E., Wilkes, J., Flammer, H., Mahler, S., … Meissner, M. (2013). An Overexpression Screen of Toxoplasma gondii Rab-GTPases Reveals Distinct Transport Routes to the Micronemes. *PLoS Pathogens*, *9*(3). doi: 10.1371/journal.ppat.1003213

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. doi: 10.1093/molbev/msi203

Kselíková, V., Singh, A., Bialevich, V., Čížková, M., & Bišová, K. (2022). Improving microalgae for biotechnology — From genetics to synthetic biology – Moving forward but not there yet. *Biotechnology Advances*, *58*. doi: https://doi.org/10.1016/j.biotechadv.2021.107885.

Kuris, A. M., Hechinger, R. F., Shaw, J. C., Whitney, K. L., Aguirre-macedo, L., Boch, C. A., … Lafferty, K. D. (2008). Ecosystem energetic implications of parasite and free-living biomass in three estuaries. *Nature*, *454*(July), 515–518. doi: 10.1038/nature06970

Kwon, H., Lu, H. L., Longnecker, M. T., & Pietrantonio, P. V. (2012). Role in Diuresis of a Calcitonin Receptor (GPRCAL1) Expressed in a Distal-Proximal Gradient in Renal Organs of the Mosquito Aedes aegypti (L.). *PLoS ONE*, *7*(11). doi: 10.1371/journal.pone.0050374

Labaude, S., Cézilly, F., De Marco, L., & Rigaud, T. (2020). Increased temperature has no consequence for behavioral manipulation despite effects on both partners in the interaction between a

crustacean host and a manipulative parasite. *Scientific Reports*, *10*(1). doi: 10.1038/s41598-020-68577-z

Laciny, A. (2021). Among the shapeshifters: parasite-induced morphologies in ants (Hymenoptera, Formicidae) and their relevance within the EcoEvoDevo framework. *EvoDevo*, *12*(1), 1–21. doi: 10.1186/s13227-021-00173-2

Laciny, A., Zettel, H., Metscher, B., Kamariah, A. S., Kopchinskiy, A., Pretzer, C., & Druzhinina, I. S. (2017). Morphological variation and mermithism in female castes of Colobopsis sp. nrSA, a Bornean "exploding ant" of the Colobopsis cylindrica group (Hymenoptera: Formicidae). *Myrmecological News*, *24*(January), 91–106.

Langfelder, P., & Horvath, S. (2008). WGCNA: An R package for weighted correlation network analysis. *BMC Bioinformatics*, *9*(559). doi: 10.1186/1471-2105-9-559

Lanzkron, S., Patrick Carroll, C., & Haywood, C. (2013). Mortality rates and age at death from sickle cell disease: U.S., 1979-2005. *Public Health Reports*, *128*(2), 110–116. doi: 10.1177/003335491312800206

Laothamatas, J., Hemachudha, T., Mitrabhakdi, E., Wannakrairot, P., & Tulayadaechanont, S. (2003). MR imaging in human rabies. *American Journal of Neuroradiology*, *24*(6), 1102–1109.

Lapierre, L. R., Gelino, S., Meléndez, A., & Hansen, M. (2011). Autophagy and lipid metabolism coordinately modulate life span in germline-less C. elegans. *Current Biology*, *21*(18), 1507–1514. doi: 10.1016/j.cub.2011.07.042

Larsen, I. S. B., Narimatsu, Y., Joshi, H. J., Siukstaite, L., Harrison, O. J., Brasch, J., … Halim, A. (2017). Discovery of an O-mannosylation pathway selectively serving cadherins and protocadherins. *Proceedings of the National Academy of Sciences of the United States of America*, *114*(42), 11163–11168. doi: 10.1073/pnas.1708319114

Latinne, A., Hu, B., Olival, K. J., Zhu, G., Zhang, L., Li, H., … Daszak, P. (2020). Origin and cross-species transmission of bat coronaviruses in China. *Nature Communications*, *11*(4235). doi: 10.1038/s41467-020-17687-3

Lavine, M. D., & Strand, M. R. (2002). Insect hemocytes and their role in immunity. *Insect Biochemistry and Molecular Biology*, *32*, 1295–1309.

Le, L., Khatoon, S., Jiménez, P., Peterson, C., Kernen, R., Zhang, W., … Siddiqui, A. A. (2020). Chronic whipworm infection exacerbates Schistosoma mansoni egg - induced hepatopathology in non - human primates. *Parasites & Vectors*, *13*(109). doi: 10.1186/s13071-020-3980-z

Lee, K. A., Kim, B., Bhin, J., Kim, D. H., You, H., Kim, E. K., … Lee, W. J. (2015). Bacterial uracil modulates drosophila DUOX-dependent Gut immunity via hedgehog-induced signaling endosomes. *Cell Host and Microbe*, *17*(2), 191–204. doi: 10.1016/j.chom.2014.12.012

Lefèvre, T., Roche, B., Poulin, R., Hurd, H., Renaud, F., & Thomas, F. (2008). Exploiting host compensatory responses: the "must" of manipulation? *Trends in Parasitology*, *24*(10), 435–439. doi: 10.1016/j.pt.2008.06.006

Leite, G. A., Pinheiro, R. T., Marcelino, D. G., Figueira, J. E. C., & Delabie, J. H. C. (2013). Foraging behavior of kaempfer's woodpecker (celeus obrieni), a bamboo specialist. *Condor*, *115*(2), 221–229. doi: 10.1525/cond.2013.120062

Lenaz, G. (1998). Role of mitochondria in oxidative stress and ageing. *Biochimica et Biophysica Acta*, *1366*, 53–67.

Leung, J. M., Graham, A. L., & Knowles, S. C. L. (2018). *Parasite-Microbiota Interactions With the Vertebrate Gut : Synthesis Through an Ecological Lens*. *9*(May), 1–20. doi: 10.3389/fmicb.2018.00843

Li, H. (2013). *Seqtk: a fast and lightweight tool for processing FASTA or FASTQ sequences*.

Li, Heng, Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., … Durbin, R. (2009). The Sequence Alignment/Map format and SAMtools. *Bioinformatics*, *25*(16), 2078–2079. doi: 10.1093/bioinformatics/btp352

Ligtenberg, W. (2019). *reactome.db: A set of annotation maps for reactome.* doi: 10.18129/B9.bioc.reactome.db

Liguori, I., Russo, G., Curcio, F., Bulli, G., Aran, L., Della-Morte, D., … Abete, P. (2018). Oxidative stress, aging, and diseases. *Clinical Interventions in Aging*, *13*, 757–772. doi: 10.2147/CIA.S158513

Lin, H., Eggesbø, M., & Peddada, S. Das. (2022). *Linear and nonlinear correlation estimators unveil undescribed taxa interactions in microbiome data*. 14–17. doi: 10.1038/s41467-022-32243-x

Lin, H., & Peddada, S. Das. (n.d.). Analysis of compositions of microbiomes with bias correction. *Nature Communications*, (c), 1–11. doi: 10.1038/s41467-020-17041-7

Lin, M. T., & Beal, M. F. (2003). The oxidative damage theory of aging. *Clinical Neuroscience Research*, *2*(5–6), 305–315. doi: 10.1016/S1566-2772(03)00007-0

Lin, N., Wu, B., Jansen, R., Gerstein, M., & Zhao, H. (2004). *Information assessment on predicting protein-protein interactions*. *11*, 1–11. doi: 10.1186/1471-2105-5-154

Lin, S. J., Defossez, P. A., & Guarente, L. (2000). Requirement of NAD and SIR2 for life-span extension by calorie restriction in saccharomyces cerevisiae. *Science*, *289*(5487), 2126–2128. doi: 10.1126/science.289.5487.2126

Linnane, A. W., Ozawa, T., Marzuki, S., & Tanaka, M. (1989). Mitochondrial DNA mutations as an important contributor to ageing and degenerative diseases. *The Lancet*, *333*(8639), 642–645.

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

Liu, K., & Czaja, M. J. (2012). Regulation of lipid stores and metabolism by lipophagy. *Cell Death and Differentiation*, *20*(1), 3–11. doi: 10.1038/cdd.2012.63

Liu, N., Wang, Y., Li, T., & Feng, X. (2021). G-protein coupled receptors (Gpcrs): Signaling pathways, characterization, and functions in insect physiology and toxicology. *International Journal of Molecular Sciences*, *22*(10), 14–18. doi: 10.3390/ijms22105260

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

Liu, Z., & Huang, X. (2013). Lipid metabolism in Drosophila: Development and disease. *Acta Biochimica et Biophysica Sinica*, *45*(1), 44–50. doi: 10.1093/abbs/gms105

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). doi: 10.1186/s13059-014-0550-8

Lu, G., Ang, Y. H., Zhou, J., Tamilarasi, J., Yan, B., Lim, Y. C., … Hooi, S. C. (2014). CCAAT/Enhancer Binding Protein a Predicts Poorer Prognosis and Prevents Energy Starvation–Induced Cell Death in Hepatocellular Carcinoma. *Hepatology*, *61*(3), 741–1099. doi: 10.1002/hep.27593

Lu, L. J., Xia, Y., Paccanaro, A., Yu, H., & Gerstein, M. (2005). *Assessing the limits of genomic data integration for predicting protein networks*. (360), 945–953. doi: 10.1101/gr.3610305.15

Luo, J., Mills, K., Cessie, S., Noordam, R., & Heemst, D. Van. (2020). Ageing , age-related diseases and oxidative stress : What to do next ? *Ageing Research Reviews*, *57*, 100982. doi: 10.1016/j.arr.2019.100982

Luz, G. F. da, Santana, W. C., Santos, C. G., Medeiros Santana, L., & Serrão, J. E. (2022). Cuticle melanization and the expression of immune-related genes in the honeybee Apis mellifera (Hymenoptera: Apidae) adult workers. *Comparative Biochemistry and Physiology Part - B: Biochemistry and Molecular Biology*, *257*(May 2021), 1–11. doi: 10.1016/j.cbpb.2021.110679

Lynch, J. B., & Hsiao, E. Y. (2019). Microbiomes as sources of emergent host phenotypes. *Science*, *365*(6460), 1405–1409. doi: 10.1126/science.aay0240

Mabbott, N. A. (2018). *The Influence of Parasite Infections on Host Immunity to Co-infection With Other Pathogens*. *9*(November), 1–14. doi: 10.3389/fimmu.2018.02579

Mackiewicz, J. S. (1988). Cestode Transmission Patterns. *The Journal of Parasitology*, *74*(1), 60–71.

Maher, P. (2005). The effects of stress and aging on glutathione metabolism. *Ageing Research Reviews*, *4*(2), 288–314. doi: 10.1016/j.arr.2005.02.005

Maksimowich, D. S., & Mathis, A. (2000). Parasitized salamanders are inferior competitors for territories and food resources. *Ethology*, *106*(4), 319–329. doi: 10.1046/j.1439-0310.2000.00526.x

Manfredini, F., Lucas, C., Nicolas, M., Keller, L., Shoemaker, D., & Grozinger, C. M. (2014). Molecular and social regulation of worker division of labour in fire ants. *Molecular Ecology*, *23*(3), 660–672. doi: 10.1111/mec.12626

Maron, J. L., & Vilà, M. (2001). When Do Herbivores Affect Plant Invasion ? Evidence for the Natural Enemies and Biotic Resistance Hypotheses. *Nordic Society Oikos*, *95*(3), 361–373.

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

Martínez-González, J. de J., Guevara-Flores, A., & Del Arenal Mena, I. P. (2022). Evolutionary Adaptations of Parasitic Flatworms to Different Oxygen Tensions. *Antioxidants*, *11*(6). doi: 10.3390/antiox11061102

Mathis, A. D., Naylor, B. C., Carson, R. H., Evans, E., Harwell, J., Knecht, J., … Price, J. C. (2017). Mechanisms of in vivo ribosome maintenance change in response to nutrient signals. *Molecular and Cellular Proteomics*, *16*(2), 243–254. doi: 10.1074/mcp.M116.063255

McArdle, A., & Jackson, M. J. (2000). Exercise, oxidative stress and ageing. *Journal of Anatomy*, *197*(4), 539–541.

McGonigle, S., Curley, G. P., & Dalton, J. P. (1997). Cloning of peroxiredoxin, a novel antioxidant enzyme, from the helminth parasite Fasciola hepatica. *Parasitology*, *115*(1), 101–104.

Mcmurdie, P. J., & Holmes, S. (2013). *phyloseq : An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data*. *8*(4). doi: 10.1371/journal.pone.0061217

Megía-Palma, R., Martínez, J., & Merino, S. (2018). Manipulation of parasite load induces significant changes in the structural-based throat color of male iberian green lizards. *Current Zoology*, *64*(3), 293–302. doi: 10.1093/cz/zox036

Mendive, F. M., Van Loy, T., Claeysen, S., Poels, J., Williamson, M., Hauser, F., … Vanden Broeck, J. (2005). Drosophila molting neurohormone bursicon is a heterodimer and the natural agonist of the orphan receptor DLGR2. *FEBS Letters*, *579*(10), 2171–2176. doi: 10.1016/j.febslet.2005.03.006

Merkwirth, C., Jovaisaite, V., Durieux, J., Matilainen, O., Sabine, D., Quiros, P. M., … Dillin, A. (2017). Two Conserved Histone Demethylases Regulate Mitochondrial Stress-Induced Longevity. *Cell*, *165*(5), 1209–1223. doi: 10.1016/j.cell.2016.04.012.Two

Metcalfe, N. B., & Alonso-Alvarez, C. (2010). Oxidative stress as a life-history constraint: The role of reactive oxygen species in shaping phenotypes from conception to death. *Functional Ecology*, *24*(5), 984–996. doi: 10.1111/j.1365-2435.2010.01750.x

Meurs, K. M., Anthony, M. A., Slater, M., & Miller, M. W. (1998). Chronic Trypanosoma cruzi infection in dogs: 11 cases (1987-1996). *Journal of the American Veterinary Medical Association*, *213*(4), 497–500.

Michener, C. D. (1964). Reproductive efficiency in relation to colony size in Hymenopterous societies. *Insectes Sociaux*, *11*(4), 317–342.

Mideo, N. (2009). Parasite adaptations to within-host competition. *Evolutionary Parasitology*, *25*(6), 261–268. doi: 10.1016/j.pt.2009.03.001

Minchella, D. J. (1985). Host life-history variation in response to parasitism. *Parasitology*, *90*(1), 205–216. doi: 10.1017/S0031182000049143

Miquel, J., Economos, A. C., Fleming, J., & Johnson, J. E. (1980). Mitochondiral role in cell aging. *Experimental Gerontology*, *15*(1974), 575–591.

Moore, J. (2002). *Parasites and the Behavior of Animals*. Oxford: Oxford University Press.

Morais, P., & Reichard, M. (2018). Cryptic invasions: A review. *Science of the Total Environment*, *613–614*, 1438–1448. doi: 10.1016/j.scitotenv.2017.06.133

Morales-Montor, J., & Larralde, C. (2005). The role of sex steroids in the complex physiology of the host-parasite relationship : the case of the larval cestode of Taenia crassiceps. *Parasitology*, *131*(3), 287–294. doi: 10.1017/S0031182005007894

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*, *139*, 153–161. doi: 10.1016/j.cca.2004.10.008

Moret, Y., & Moreau, J. (2012). The immune role of the arthropod exoskeleton. *Invertebrate Survival Journal*, *9*(2), 200–206.

Mouritsen, K. N., & Poulin, R. (2002). Parasitism, community structure and biodiversity in intertidal ecosystems. *Parasitology*, *124*(SUPPL.). doi: 10.1017/s0031182002001476

Muir, D. A. (1954). Ants Myrmica rubra L. and M. scabrinodis Nylander as Intermediate Hosts of a Cestode. *Nature*, *173*, 688–689.

Munang'andu, H. M. (2018). Intracellular bacterial infections: A challenge for developing cellular mediated immunity vaccines for farmed fish. *Microorganisms*, *6*(2). doi: 10.3390/microorganisms6020033

Naguleswaran, A., Alaeddine, F., Guionaud, C., Vonlaufen, N., Sonda, S., Jenoe, P., … Hemphill, A. (2005). Neospora caninum protein disulfide isomerase is involved in tachyzoite-host cell interaction. *International Journal for Parasitology*, *35*(13), 1459–1472. doi: 10.1016/j.ijpara.2005.06.006

Nappi, A. J., & Vas, E. (1993). Melanogenesis and the Generation of Cytotoxic Molecules During Insect Cellular Immune Reactions. *Pigment Cell Research*, *6*, 117–126.

Nargund, A., Pellegrino, M., Fiorese, C., Baker, B., & Haynes, C. (2012). Mitochondrial Import Efficiency of AFTS-1 Regulates Mitochondrial UPR Activation. *Science*, *337*(6094), 587–590. doi: 10.1126/science.1223560

Naug, D., & Wenzel, J. (2006). Constraints on foraging success due to resource ecology limit colony productivity in social insects. *Behavioral Ecology and Sociobiology*, *60*(1), 62–68. doi: 10.1007/s00265-005-0141-5

Nawab, N., Ara, A. J., Kumar, P. A., Pankaj, G., & Rezzaque, C. A. (2016). Cestodiasis in scaly-bellied woodpeckers (Picus squamatus) Of Kashmir. *Bhartiya Krishi Anusandhan Patrika*, *31*(1).

Negroni, M. A., Foitzik, S., & Feldmeyer, B. (2019). Long-lived Temnothorax ant queens switch from investment in immunity to antioxidant production with age. *Scientific Reports*, *9*(1), 1–10. doi: 10.1038/s41598-019-43796-1

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*(1), 1–16. doi: 10.1186/s12864-020-07191-9

Nguyen, V. B., & Wang, S.-L. (2017). Reclamation of Marine Chitinous Materials for the Production of α -Glucosidase Inhibitors via. *Marine Drugs*, *15*(11). doi: 10.3390/md15110350

Nikooie, R., Jafari-Sardoie, S., Sheibani, V., & Chatroudi, A. N. (2020). Resistance training - induced muscle hypertrophy is mediated by TGF-β 1-Smad signaling pathway in male Wistar rats. *Journal of Cellular Physiology*, *235*(7–8), 5649–5665. doi: 10.1002/jcp.29497

Nishizuka, Y. (1980). Metabolic Maps. *Tokyo Kagaku Dojin*.

Noël, W., Raes, G., Ghassabeh, G. H., De Baetselier, P., & Beschin, A. (2004). Alternatively activated macrophages during parasite infections. *Trends in Genetics*, *20*(3), 126–133. doi: 10.1016/j.pt.2004.01.004

Novak, M., Modha, A., & Blackburn, B. J. (1993). d-[1-13C]Glucose Metabolism of Tribolium confusum Parasitized by Hymenolepid Metacestodes. *Journal of Invertebrate Pathology*, *62*(3), 302–307.

O'Rourke, E. J., Kuballa, P., Xavier, R., & Ruvkun, G. (2013). ω-6 Polyunsaturated fatty acids extend life span through the activation of autophagy. *Genes and Development*, *27*(4), 429–440. doi: 10.1101/gad.205294.112

O'Shea-Wheller, T. A., Hunt, E. R., & Sasaki, T. (2021). Functional heterogeneity in superorganisms:

Emerging trends and concepts. *Annals of the Entomological Society of America*, *114*(5), 562–574. doi: 10.1093/aesa/saaa039

Odum, E. P., & Barrett, G. W. (1971). *Fundamentals of Ecology* (5th ed.). Philadelphia, Pennsylvania, USA.: 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.

Okada, H., Kuhn, C., Feillet, H., & Bach, J. F. (2010). The "hygiene hypothesis" for autoimmune and allergic diseases: An update. *Clinical and Experimental Immunology*, *160*(1), 1–9. doi: 10.1111/j.1365-2249.2010.04139.x

Olano, J. P., Weller, P. F., Guerrant, R. L., & Walker, D. H. (2011). Principles of Parasitism : Host – Parasite Interactions. In *Tropical Infectious Diseases: Principles, Pathogens and Practice.* doi: 10.1016/B978-0-7020-3935-5.00001-X

Onchuru, T. O., Martinez, A. J., & Kaltenpoth, M. (2018). The cotton stainer's gut microbiota suppresses infection of a cotransmitted trypanosomatid parasite. *Molecular Ecology*, *27*(16), 3408–3419. doi: 10.1111/mec.14788

Orr, T. S. C. (1966). Spawning behaviour of rudd, Scardinius erythrophthalmus infested with plerocercoids of Ligula intestinalis. *Nature*, *212*(5063), 736. doi: 10.1038/212736a0

Paine, R. T. (1966). Food Web Complexity and Species Diversity. *The American Naturalist*, *100*(910), 65–75.

Palomares, M. A., Dalmasso, C., Bonnet, E., Derbois, C., Brohard-Julien, S., Ambroise, C., … Olaso, R. (2019). Systematic analysis of TruSeq, SMARTer and SMARTer Ultra-Low RNA-seq kits for standard, low and ultra-low quantity samples. *Scientific Reports*, *9*(1), 1–12. doi: 10.1038/s41598-019-43983-0

Paludo, G. P., Thompson, C. E., Miyamoto, K. N., Guedes, R. L. M., Zaha, A., De Vasconcelos, A. T. R., … Ferreira, H. B. (2020). Cestode strobilation: Prediction of developmental genes and pathways. *BMC Genomics*, *21*(1), 1–16. doi: 10.1186/s12864-020-06878-3

Pappas, G., Kiriaze, I. J., & Falagas, M. E. (2008). *Insights into infectious disease in the era of Hippocrates*. doi: 10.1016/j.ijid.2007.11.003

Parker, G. A., Chubb, J. C., Ball, M. A., & Roberts, G. N. (2003). Evolution of complex life cycles in helminth parasites. *Nature*, *425*, 480–484.

Parker, J. D., Parker, K. M., Sohal, B. H., & Sohal, R. S. (2004). Decreased expression of Cu-Zn superoxide dismutase 1 in ants with extreme lifespan. *Proceedings of the National Academy of Sciences of the United States of America*, *101*(10), 3486–3489. doi: 10.1073/pnas.0400222101

Parratt, S. R., & Laine, A. (2016). The role of hyperparasitism in microbial pathogen ecology and evolution. *Nature Publishing Group*, *10*(8), 1815–1822. doi: 10.1038/ismej.2015.247

Parratt, S. R., & Laine, A. L. (2018). Pathogen dynamics under both bottom-up host resistance and top-down hyperparasite attack. *Journal of Applied Ecology*, *55*(6), 2976–2985. doi: 10.1111/1365-2664.13185

Parthasarathy, A., Cross, P. J., Dobson, R. C. J., Adams, L. E., Savka, M. A., & Hudson, A. O. (2018). A

Three-Ring circus: Metabolism of the three proteogenic aromatic amino acids and their role in the health of plants and animals. *Frontiers in Molecular Biosciences*, *5*(APR), 1–30. doi: 10.3389/fmolb.2018.00029

Paskewitz, S. M., & Gorman, M. J. (1999). Mosquito Immunity and Malaria parasites. *American Entomologist*, *45*(2), 80–94.

Patel, S. (2017). A critical review on serine protease: Key immune manipulator and pathology mediator. *Allergologia et Immunopathologia*, *45*(6), 579–591. doi: 10.1016/j.aller.2016.10.011

Pawelec, T. F. A. L. G., Cohen, A. K. A. A., & Witkowski, K. H. J. M. (2023). Immunology of Aging: the Birth of Inflammaging. *Clinical Reviews in Allergy & Immunology*, *64*(2), 109–122. doi: 10.1007/s12016-021-08899-6

Pedrajas, J. R., Carreras, A., Valderrama, R., & Barroso, J. B. (2010). Mitochondrial 1-Cys-peroxiredoxin/thioredoxin system protects manganese-containing superoxide dismutase (Mn-SOD) against inactivation by peroxynitrite in Saccharomyces cerevisiae. *Nitric Oxide - Biology and Chemistry*, *23*(3), 206–213. doi: 10.1016/j.niox.2010.06.004

Pelletier, F., & Festa-bianchet, M. (2004). Effects of body mass, age, dominance and parasite load on foraging time of bighorn rams, Ovis canadensis. *Behavioral Ecology and Sociobiology*, *56*, 546–551. doi: 10.1007/s00265-004-0820-7

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). doi: 10.1098/rsos.171332

Pertea, G., & Pertea, M. (2023). *GFF Utilities : GffRead and GffCompare [version 2 ; peer review : 3 approved]*. 1–20.

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. doi: 10.1038/nbt.3122

Piel, F. B., Patil, A. P., Howes, R. E., Nyangiri, O. A., Gething, P. W., Dewi, M., … Hay, S. I. (2013). Global epidemiology of Sickle haemoglobin in neonates: A contemporary geostatistical model-based map and population estimates. *The Lancet*, *381*(9861), 142–151. doi: 10.1016/S0140-6736(12)61229-X

Piel, F. B., Patil, A. P., Howes, R. E., Nyangiri, O. A., Gething, P. W., Williams, T. N., … Hay, S. I. (2010). Global distribution of the sickle cell gene and geographical confirmation of the malaria hypothesis. *Nature Communications*, *1*(104). doi: 10.1038/ncomms1104

Pierson, L., Mousley, A., Devine, L., Marks, N. J., Day, T. A., & Maule, A. G. (2010). RNA interference in a cestode reveals specific silencing of selected highly expressed gene transcripts. *International Journal for Parasitology*, *40*(5), 605–615. doi: 10.1016/j.ijpara.2009.10.012

Pirovino, M., Iseli, C., Curran, J. A., & Conrad, B. (2023). Birth of immunity in early life: stepwise emergence of resistance and immunity to complex molecular parasites via progressively degenerating hyperparasites. *BioRxiv*.

Plateaux, L. (1972). Sur les modifications produites chez une fourmi par la présence d'un parasite cestode. *Annales Des Sciences Naturelles*, *2*(14).

Pleass, R. J., Kusel, J. R., & Woof, J. M. (2000). Cleavage of Human IgE Mediated by Schistosoma mansoni. *Allergy and Immunology*, *121*, 194–204.

Poinar, G. (2012). Nematode parasites and associates of ants: Past and present. *Psyche*, *2012*. doi: 10.1155/2012/192017

Ponton, F., Otálora-Luna, F., Lefèvre, T., Guerin, P. M., Lebarbenchon, C., Duneau, D., … Thomas, F. (2011). Water-seeking behavior in worm-infected crickets and reversibility of parasitic manipulation. *Behavioral Ecology*, *22*(2), 392–400. doi: 10.1093/beheco/arq215

Poulin, R., Bennett, J., Dutra, D. D. A., & Doherty, J. (2020). Evolutionary Signature of Ancient Parasite Pressures , or the Ghost of Parasitism Past. *Frontiers in Ecology and Evolution*, *8*. doi: 10.3389/fevo.2020.00195

Poulin, R., Brodeur, J., & Moore, J. (1994). Parasite Manipulation of Host Behaviour: Should Hosts Always Lose? *Oikos*, *70*(3), 479–484.

Poulin, R., & Maure, F. (2015). Host Manipulation by Parasites: A Look Back Before Moving Forward. *Trends in Parasitology*, *31*(11), 563–570. doi: 10.1016/j.pt.2015.07.002

Poulin, R., & Morand, S. (2000). The diversity of parasites. *Thle Quarterly Review of Biology*, *75*(3). Retrieved from http://www.journals.uchicago.edu/t-and-c

Poulin, R., & Thomas, F. (1999). Phenotypic variability induced by parasites: Extent and evolutionary implications. *Parasitology Today*, *15*(1), 28–32. doi: 10.1016/S0169-4758(98)01357-X

Powers, S. K., Ji, L. L., Kavazis, A. N., & Jackson, M. J. (2011). Reactive oxygen species: impact on skeletal muscle. *Comprehensive Physiology*, *1*(2), 941–969. doi: 10.1002/cphy.c100054.REACTIVE

Prebus, M. (2017). Insights into the evolution, biogeography and natural history of the acorn ants, genus Temnothorax Mayr (hymenoptera: Formicidae). *BMC Evolutionary Biology*, *17*(1), 1–22. doi: 10.1186/s12862-017-1095-8

Preston, D. L., Orlofske, S. A., Lambden, J. P., & Johnson, P. T. J. (2013). *Biomass and productivity of trematode parasites in pond ecosystems*. 509–517. doi: 10.1111/1365-2656.12030

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.

Rahman, M., Haberman, A., Tracy, C., Ray, S., & Krämer, H. (2012). Drosophila mauve mutants reveal a role of LYST homologs late in the maturation of phagosomes and autophagosomes. *Traffic*, *13*(12), 1680–1692. doi: 10.1111/tra.12005

Rahnenfuhrer, A. (2022). *topGO: Enrichment Analysis for Gene Ontology. R package version 2.50.0.*

Ramachandran, P. V., Savini, M., Folick, A. K., Hu, K., Masand, R., Graham, B. H., & Wang, M. C. (2019). Lysosomal Signaling Promotes Longevity by Adjusting Mitochondrial Activity. *Developmental Cell*, *48*(5), 685–696. doi: 10.1016/j.devcel.2018.12.022

Ramanan, D., Bowcutt, R., Lee, S. C., Tang, M. S., Kurtz, Z. D., Ding, Y., … Cadwell, K. (2016). *Helminth infection promotes colonization resistance via type 2 immunity*. *352*(6285), 608–613.

Ranson, H., & Hemingway, J. (2005). Mosquito glutathione transferases. *Methods in Enzymology*, *401*(1977), 226–241. doi: 10.1016/S0076-6879(05)01014-1

Reddy, K. C., Dunbar, T. L., Nargund, A. M., Haynes, C. M., Emily, R., Diego, C. S., … Program, B. (2016).

The C. elegans CCAAT-enhancer binding protein gamma is required for surveillance immunity. *Cell Reports*, *14*(7), 1581–1589. doi: 10.1016/j.celrep.2016.01.055.The

Redi, F. (1668). *Esperienze intorno alla generazione degl'insetti*.

Reece, S. T., & Kaufmann, S. H. E. (2019). Host Defenses to Intracellular Bacteria. In *Clinical Immunology* (5th ed., pp. 375–389). Elsevier.

Regan, L. O., Steinfelder, S., Venugopal, G., Rao, G. B., Lucius, R., Srikantam, A., & Hartmann, S. (2014). *Brugia malayi Microfilariae Induce a Regulatory Monocyte / Macrophage Phenotype That Suppresses Innate and Adaptive Immune Responses*. *8*(10). doi: 10.1371/journal.pntd.0003206

Reid, A. J., & Berriman, M. (2013). Genes involved in host – parasite interactions can be revealed by their correlated expression. *Nucleic Acids Research*, *41*(3), 1508–1518. doi: 10.1093/nar/gks1340

Reid, W. M., & Nugara, D. (1961). Description and Life Cycle of Raillietina georgiensis n. sp., a Tapeworm from Wild and Domestic Turkeys. *The Journal of Parasitology*, *47*(6), 885–889.

Ristow, M., & Schmeisser, S. (2011). Extending life span by increasing oxidative stress. *Free Radical Biology and Medicine*, *51*(2), 327–336. doi: 10.1016/j.freeradbiomed.2011.05.010

Roh, K., Kim, C., Lee, H., Kwon, H., Park, J., Ryu, J., … So, K. (2009). Proteolytic Cascade for the Activation of the Insect Toll Pathway Induced by the Fungal Cell Wall Component □. *The Journal of Biological Chemistry*, *284*(29), 19474–19481. doi: 10.1074/jbc.M109.007419

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

Roode, Jacobus C De, Lefèvre, T., & Hunter, M. D. (2013). Self-Medication in Animals. *Science*, *340*(6129), 150–151.

Round, J. L., & Mazmanian, S. K. (2009). The gut microbiota shapes intestinal immune responses during health and disease. *Nature Reviews Immunology*, *9*(5), 313–323. doi: 10.1038/nri2515

Salinas, G., Fernandez, V., Fernandez, C., & Selkirk, M. E. (1998). Echinococcus granulosus: Cloning of a Thioredoxin Peroxidase. *Experimental Parasitology*, *90*, 298–301.

Sánchez, M. I., Pons, I., Martínez-Haro, M., Taggart, M. A., Lenormand, T., & Green, A. J. (2016). When Parasites Are Good for Health: Cestode Parasitism Increases Resistance to Arsenic in Brine Shrimps. *PLoS Pathogens*, *12*(3), 1–19. doi: 10.1371/journal.ppat.1005459

Sarant, L. (2017). Egypt: The flatworm's revenge. *Nature*, *551*. doi: https://doi.org/10.1038/551S46a

Savino, W. (2006). The thymus is a common target organ in infectious diseases. *PLoS Pathogens*, *2*(6), 0472–0483. doi: 10.1371/journal.ppat.0020062

Scanlan, J. L., Gledhill-Smith, R. S., Battlay, P., & Robin, C. (2020). Genomic and transcriptomic analyses in Drosophila suggest that the ecdysteroid kinase-like (EcKL) gene family encodes the 'detoxification-by-phosphorylation' enzymes of insects. *Insect Biochemistry and Molecular Biology*, *123*, 103429. doi: 10.1016/j.ibmb.2020.103429

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

Schmid-hempel, P. (2008). Parasite immune evasion : a momentous molecular war. *Trends in Ecology and Evolution*, *23*(6), 318–326. doi: 10.1016/j.tree.2008.02.011

Schmid-hempel, P. (2009). *Immune defence , parasite evasion strategies and their relevance for ' macroscopic phenomena ' such as virulence*. (October 2008), 85–98. doi: 10.1098/rstb.2008.0157

Schmidt, R. L., Trejo, T. R., Plummer, T. B., Platt, J. L., & Tang, A. H. (2008). Infection-induced proteolysis of PGRP-LC controls the IMD activation and melanization cascades in Drosophila. *The FASEB Journal*, *22*, 918–929. doi: 10.1096/fj.06-7907com

Schultheiss, P., Nooten, S. S., Wang, R., Wong, M. K. L., Brassard, F., & Guénard, B. (2022). The abundance, biomass, and distribution of ants on Earth. *Proceedings of the National Academy of Sciences of the United States of America*, *119*(40), 1–9. doi: 10.1073/pnas.2201550119

Scott, J. M. C. A. L. (2017). Antecedent Nippostrongylus infection alters the lung immune response to Plasmodium berghei. *Parasite Immunology*, *39*(8). doi: 10.1111/pim.12441

Segers, F. H. I. D., Kaltenpoth, M., & Foitzik, S. (2019). Abdominal microbial communities in ants depend on colony membership rather than caste and are linked to colony productivity. *Ecology and Evolution*, *9*(23), 13450–13467. doi: 10.1002/ece3.5801

Seifert, R., & Wenzel-Seifert, K. (2002). Constitutive activity of G-proteins-coupled receptors: Cause of disease and common property of wild-type receptors. *Naunyn-Schmiedeberg's Archives of Pharmacology*, *366*(5), 381–416. doi: 10.1007/s00210-002-0588-0

Shannon, P., Markiel, A., Ozier, O., Baliga, N. S., Wang, J. T., Ramage, D., … Ideker, T. (2003). Cytoscape: A Software Environment for Integrated Models. *Genome Research*, *13*(22), 426. doi: 10.1101/gr.1239303.metabolite

Shaw, P. J., Cirelli, C., Greenspan, R. J., & Tononi, G. (2000). Correlates of sleep and waking in Drosophila melanogaster. *Science*, *287*(5459), 1834–1837. doi: 10.1126/science.287.5459.1834

Shin, H. R., Ko, E. J., Kang, Y. J., Yu, H. S., Ock, M. S., & Cha, H. J. (2023). Effect of parasitic infection on muscular function of dystrophin gene (Dmd) deficient mouse. *Genes and Genomics*, *45*(2), 183–190. doi: 10.1007/s13258-022-01355-5

Sidman, R. L., Taksir, T., Fidler, J., Zhao, M., James, C., Passini, M. A., … Shihabuddin, L. S. (2009). Temporal Neuropathological and Behavioral Phenotype of 6neo/6neo Pompe Disease Mice. *Journal of Neuropathology and Experimental Neurology*, *67*(8), 803–818. doi: 10.1097/NEN.0b013e3181815994.Temporal

Siles-Lucas, M., Morchon, R., Simon, F., & Manzano-Roman, R. (2015). Exosome-transported microRNAs of helminth origin: New tools for allergic and autoimmune diseases therapy? *Parasite Immunology*, *37*(4), 208–214. doi: 10.1111/pim.12182

Simon, M. A., Drees, B., Kornberg, T., & Bishop, J. M. (1985). The nucleotide sequence and the tissue-specific expression of Drosophila c-src. *Cell*, *42*(3), 831–840. doi: 10.1016/0092-8674(85)90279-X

Sinclair, A. R. E. (1979). The eruption of the ruminants. In A. R. E. Sinclair & N. Norton-Griffiths (Eds.), *In Serengetti: Dynamics of an Ecosystem* (pp. 82–103). Chicago: 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*, (February), 4412–4426. doi: 10.1111/mec.16995

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

Smith, C. R., Anderson, K. E., Tillberg, C. V, Gadau, J., & Suarez, A. V. (2008). Caste Determination in a Polymorphic Social Insect: Nutritional, Social, and Genetic Factors. *The American Naturalist*, *172*(4), 497–507. doi: 10.1086/590961

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. doi: 10.1016/S0166-6851(96)02615-1

Smyth, J. D. (1946). Studies on tapeworm physiology, the cultivation of Schistocephalus solidus in vitro. *The Journal of Experimental Biology*, *23*(1), 47–70.

Sohal, R. S., & Weindruch, R. (1996). Oxidative stress, caloric restriction, and aging. *Science*, *273*(5271), 59–63. doi: 10.1126/science.273.5271.59

Sokolova, N., Pan, S., Provazza, S., Beutner, G., Vendelin, M., & Birkedal, R. (2013). ADP Protects Cardiac Mitochondria under Severe Oxidative Stress. *PloS One*, *8*(12). doi: 10.1371/journal.pone.0083214

Sopko, R., & Perrimon, N. (2013). Receptor tyrosine kinases in Drosophila development. *Cold Spring Harbor Perspectives in Biology*, *5*(6). doi: 10.1101/cshperspect.a009050

Soyemi, J., Isewon, I., Oyelade, J., & Adebiyi, E. (2018). *Inter-Species/Host-Parasite Protein Interaction Predictions Reviewed*. 396–406. doi: 10.2174/1574893613666180108155851

Spottiswoode, C. N., & Busch, R. (2019). Vive la difference ! Self/non-self recognition and the evolution of signatures of identity in arms races with parasites. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *374*. doi: 10.1098/rstb.2018.0206

Srinivasan, S., & Avadhani, N. G. (2012). Cytochrome c oxidase dysfunction in oxidative stress. *Free Radical Biology and Medicine*, *53*(6), 1252–1263. doi: 10.1016/j.freeradbiomed.2012.07.021

Stamatoyannopoulos, G. (1972). The molecular basis of hemoglobin disease. *Annual Review of Genetics*, (165), 47–70.

Stegehake, D., Daniel, J., Drescher, M., Ajonina, I., Ajonina, C., Hertel, P., … Liebau, E. (2013). The Glutathione Reductase GSR-1 Determines Stress Tolerance and Longevity in Caenorhabditis elegans. *PLoS ONE*, *8*(4). doi: 10.1371/journal.pone.0060731

Stiemsma, L. T., Reynolds, L. A., Turvey, S. E., & Finlay, B. B. (2015). *The hygiene hypothesis : current perspectives and future therapies*. 143–157.

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

Stocker, R., Hunt, N. H., Buffinton, G. D., Weidemann, M. J., Lewis-Hughes, P. H., & Clark, I. A. (1985). Oxidative stress and protective mechanisms in erythrocytes in relation to Plasmodium vinckei load. *Proceedings of the National Academy of Sciences of the United States of America*, *82*(2), 548–551. doi: 10.1073/pnas.82.2.548

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). doi: 10.3390/genes12010095

Stoldt, M., Negroni, M. A., Feldmeyer, B., & Foitzik, S. (2024). Molecular adjustment to a social niche:

Brain transcriptomes reveal divergent influence of social environment on the two queen morphs of the ant Temnothorax rugatulus. *Unknown*.

Stolf, B. S., Smyrnias, I., Lopes, L. R., Vendramin, A., Goto, H., Laurindo, F. R. M., … Santos, C. X. C. (2011). Protein disulfide isomerase and host-pathogen interaction. *TheScientificWorldJournal*, *11*, 1749–1761. doi: 10.1100/2011/289182

Strand, M. R., & Pech, L. L. (1995). Immunological basis for compatibility in parasitoid-host relationships. *Annual Review of Entomology*, *40*, 31–56.

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

Su, J., Li, F., Wang, Y., Su, Y., Verhaar, A., Ma, Z., & Peppelenbosch, M. P. (2022). Investigating Ramadan Like Fasting Effects on the Gut Microbiome in BALB / c Mice. *Frontiers in Microbiology*, *9*. doi: 10.3389/fnut.2022.832757

Sugden, K., Moffitt, T. E., Pinto, L., Poulton, R., Williams, B. S., & Caspi, A. (2016). Is Toxoplasma Gondii Infection related to brain and behavior impairments in humans? Evidence from a population-representative birth cohort. *PLoS ONE*, *11*(2), 1–14. doi: 10.1371/journal.pone.0148435

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. doi: 10.1007/s11738-012-0935-9

Sukhdeo, M. (1992). The Behavior of Parasitic Flatworms In vivo: What Is the Role of the Brain ? *The Journal of Parasitology*, *78*(2), 231–242.

Tasaki, E., Kobayashi, K., Matsuura, K., & Iuchi, Y. (2018). Long-Lived Termite Queens Exhibit High Cu/Zn-Superoxide Dismutase Activity. *Hindawi*.

Tay, M. Z., Poh, C. M., Rénia, L., Macary, P. A., & Ng, L. F. P. (2020). The trinity of COVID-19: immunity, inflammation and intervention. *Nature Reviews Immunology*, *20*(June), 363–374. doi: 10.1038/s41577-020-0311-8

Terhzaz, S., Cabrero, P., Robben, J. H., Radford, J. C., Hudson, B. D., Milligan, G., … Davies, S. A. (2012). Mechanism and function of drosophila capa GPCR: A desiccation stress-responsive receptor with functional homology to human neuromedinu receptor. *PLoS ONE*, *7*(1). doi: 10.1371/journal.pone.0029897

Thakur, A., Mikkelsen, H., & Jungersen, G. (2019). Intracellular pathogens: Host immunity and microbial persistence strategies. *Journal of Immunology Research*, *2019*. doi: 10.1155/2019/1356540

Thomas, F., & Bonsall, M. B. (2005). Parasitism, biodiversity and conservation. In F. Thomas & F. Renaud (Eds.), *Parasitism and Ecosystems* (pp. 124–139). New York: Oxford University Press.

Thompson, A. A. R., Dickinson, R. S., Murphy, F., Thomson, J. P., Marriott, H. M., Tavares, A., … Walmsley, S. R. (2017). Hypoxia determines survival outcomes of bacterial infection through HIF-1α–dependent reprogramming of leukocyte metabolism. *Science Immunology*, *2*(8). doi: 10.1126/sciimmunol.aal2861

Thompson, S. N. (2003). Trehalose - The Insect “Blood” Sugar. *Advances in Insect Physiology*, *31*. doi: 10.1016/S0065-2806(03)31004-5

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. doi: 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. doi: 10.1007/s10695-015-0045-x

Tokuhiro, K., Ikawa, M., Benham, A. M., & Okabe, M. (2012). Protein disul fi de isomerase homolog PDILT is required for quality control of sperm membrane protein ADAM3 and male fertility. *Proceedings of the National Academy of Sciences of the United States of America*, *109*(10), 3850–3855. doi: 10.1073/pnas.1117963109

Toprak, U. (2020). The Role of Peptide Hormones in Insect Lipid Metabolism. *Frontiers in Physiology*, *11*, 1–33. doi: 10.3389/fphys.2020.00434

Trabalon, M., Plateaux, L., Péru, L., Bagnères, A. G., & Hartmann, N. (2000). Modification of morphological characters and cuticular compounds in worker ants Leptothorax nylanderi induced by endoparasites Anomotaenia brevis. *Journal of Insect Physiology*, *46*(2), 169–178. doi: 10.1016/S0022-1910(99)00113-4

Tritten, L., & Geary, T. G. (2018). Helminth extracellular vesicles in host–parasite interactions. *Current Opinion in Microbiology*, *46*, 73–79. doi: 10.1016/j.mib.2018.08.002

Tsirogianni, A. K., Moutsopoulos, N. M., & Moutsopoulos, H. M. (2006). Wound healing: Immunological aspects. *Injury*, *37*(1). doi: 10.1016/j.injury.2006.02.035

Van Riet, E., Hartgers, F. C., & Yazdanbakhsh, M. (2007). Chronic helminth infections induce immunomodulation : Consequences and mechanisms. *Immunobiology*, *212*, 475–490. doi: 10.1016/j.imbio.2007.03.009

Vantaux, A., Yao, F., Hien, D. F., Yameogo, B. K., Gouagna, L.-C., Fontenille, D., … Lefèvre, T. (2021). Peer Community Journal. *Peer Community Journal*, *1*. doi: 10.24072/pcjournal.13

Vardo, A. M., Kaufhold, K. D., & Schall, J. J. (2007). Experimental test for premonition in a lizard malaria parasite (Plasmodium mexicanum). *Journal of Parasitology*, *93*(2), 280–282. doi: 10.1645/GE-1005R.1

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.

Vendelova, E., Lutz, M. B., & Hrčková, G. (2015). Immunity and immune modulation elicited by the larval cestode Mesocestoides vogae and its products. *Parasite Immunology*, *37*(10), 493–504. doi: 10.1111/pim.12216

Vilar-Pereira, G., Barrios, L. C., da Silva, A. A., MartinsBatista, A., Pereira, I. R., Moreira, O. C., … Lannes-Vieira, J. (2021). Memory impairment in chronic experimental Chagas disease: Benznidazole therapy reversed cognitive deficit in association with reduction of parasite load and oxidative stress in the nervous tissue. *PLoS ONE*, *16*(1 January 2021), 1–21. doi: 10.1371/journal.pone.0244710

Voigt, B., Schweder, T., Sibbald, M. J. J. B., Albrecht, D., Ehrenreich, A., Bernhardt, J., … Hecker, M. (2006). The extracellular proteome of Bacillus licheniformis grown in different media and under different nutrient starvation conditions. *Proteomics*, *6*, 268–281. doi: 10.1002/pmic.200500091

Vuitton, D. A. (2003). The ambiguous role of immunity in echinococcosis : protection of the host or of the parasite? *Acta Tropica*, *85*, 119–132.

Vysotskaya, R. U., Krupnova, M. Y., Ieshko, E. P., Anikieva, L. V., & Lebedeva, D. I. (2015). Ecological and biochemical aspects of parasite-host interactions in transformed aquatic bodies: A case study of the cestode Triaenophorus nodulosus and its host, the northern pike Esox lucius. *Biology Bulletin*, *42*(3), 246–253. doi: 10.1134/S1062359015030140

Walderdorff, L., Laval-Gilly, P., Bonnefoy, A., & Falla-Angel, J. (2018). Imidacloprid intensifies its impact on honeybee and bumblebee cellular immune response when challenged with LPS (lippopolysacharide) of Escherichia coli. *Journal of Insect Physiology*, *108*(November 2017), 17–24. doi: 10.1016/j.jinsphys.2018.05.002

Wang, H., Zhang, C. S., Fang, B. Bin, Li, Z. De, Li, L., Bi, X. J., … Wen, H. (2019). Thioredoxin peroxidase secreted by Echinococcus granulosus (sensu stricto) promotes the alternative activation of macrophages via PI3K//AKT/mTOR pathway. *Parasites & Vectors*, *12*(542). doi: 10.1186/s13071-019-3786-z

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. doi: 10.1038/nature11832

Wang, L., Wu, J., Li, K., Sadd, B. M., Guo, Y., Zhuang, D., … Chen, Y. (2019). Dynamic Changes of Gut Microbial Communities of Bumble Bee Queens through Important Life Stages. *Host-Microbe Biology*, *4*(6).

Watanabe, D., Gotoh, H., Miura, T., & Maekawa, K. (2014). Social interactions affecting caste development through physiological actions in termites. *Frontiers in Ph*, *5*(April). doi: 10.3389/fphys.2014.00127

Webb, R. A., & Mettrick, D. F. (1975). The role of glucose in the lipid metabolism of the rat tapeworm Hymenolepis diminuta. *International Journal for Parasitology*, *5*(1), 107–112. doi: 10.1016/0020-7519(75)90107-1

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. doi: 10.1007/s10393-017-1276-3

Wedekind, C., Strahm, D., & Schärer, L. (1998). Evidence for strategic egg production in a hermaphroditic cestode. *Parasitology*, *117*(4), 373–382. doi: 10.1017/S0031182098003114

Weinersmith, K., & Faulkes, Z. (2014). Parasitic manipulation of hosts' phenotype, or how to make a zombie - An introduction to the symposium. *Integrative and Comparative Biology*, *54*(2), 93–100. doi: 10.1093/icb/icu028

Weinreich, F., Kalbe, M., & Benesh, D. P. (2014). Making the in vitro breeding of Schistocephalus solidus more flexible. *Experimental Parasitology*, *139*(1), 1–5. doi: 10.1016/j.exppara.2014.02.002

White, E. C., Houlden, A., Bancroft, A. J., Hayes, K. S., Goldrick, M., Grencis, R. K., & Roberts, I. S.

(2018a). *Manipulation of host and parasite microbiotas : Survival strategies during chronic nematode infection*. (March), 1–11.

White, E. C., Houlden, A., Bancroft, A. J., Hayes, K. S., Goldrick, M., Grencis, R. K., & Roberts, I. S. (2018b). Manipulation of host and parasite microbiotas: Survival strategies during chronic nematode infection. *Science Advances*, *4*(3), 1–10. doi: 10.1126/sciadv.aap7399

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. doi: 10.1006/bbrc.2001.5209

Wickersham, M., Wachtel, S., Wong Fok Lung, T., Soong, G., Jacquet, R., Richardson, A., … Prince, A. (2017). Metabolic Stress Drives Keratinocyte Defenses against Staphylococcus aureus Infection. *Cell Reports*, *18*(11), 2742–2751. doi: 10.1016/j.celrep.2017.02.055

Wilson, E. O. (1971). *The Insects Societies*. Cambridge, Massachusetts: Harvard University Press.

Windsor, D. A. (1998). Most of the species on Earth are parasites. *International Journal for Parasitology*, *28*(12), 1939–1941. doi: 10.1016/S0020-7519(98)00153-2

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), 1–13. doi: 10.12688/f1000research.15931.1

Wood, C. L., & Johnson, P. T. J. (2015). A world without parasites: exploring the hidden ecology of infection. *Frontiers in Ecology and the Environment*, *13*(8), 425–434. doi: 10.1890/140368

Wood, C. L., Welicky, R. L., Preisser, W. C., Leslie, K. L., Mastick, N., Greene, C., … Essington, T. E. (2023). A reconstruction of parasite burden reveals one century of climate-associated parasite decline. *PNAS*, *120*(3), 1–9. doi: 10.1073/pnas.

World Health Organisation (2022). *World malaria report 2022*. Geneva.

Wronska, A. K., Kaczmarek, A., Bogus, M. I., & Kuna, A. (2023). Lipids as a key element of insect defense systems. *Frontiers in Genetics*, *14*. doi: 10.3389/fgene.2023.1183659

Wu, T., Hu, E., Xu, S., Chen, M., Guo, P., Dai, Z., … Yu, G. (2021). clusterProfiler 4.0: A universal enrichment tool for interpreting omics data. *The Innovation*, *2*(3), 100141. doi: 10.1016/j.xinn.2021.100141

Xiao, X., Huang, M., Fan, C., & Zuo, F. (2021). DUOX2 participates in skin aging induced by UVB in HSF2 cells by activating NF - κ B signaling. *Experimental and Therapeutic Medicine*, *21*(157), 1–9. doi: 10.3892/etm.2020.9588

Yan, J., Martínez-de la Puente, J., Gangoso, L., Gutiérrez-López, R., Soriguer, R., & Figuerola, J. (2018). Avian malaria infection intensity influences mosquito feeding patterns. *International Journal for Parasitology*, *48*(3–4), 257–264. doi: 10.1016/j.ijpara.2017.09.005

Yang, W., Li, J., & Hekimi, S. (2007). A measurable increase in oxidative damage due to reduction in superoxide detoxification fails to shorten the life span of long-lived mitochondrial mutants of Caenorhabditis elegans. *Genetics*, *177*(4), 2063–2074. doi: 10.1534/genetics.107.080788

Yanoviak, S. P., Kaspari, M., Dudley, R., & Poinar, G. (2008). Parasite-induced fruit mimicry in a tropical canopy ant. *American Naturalist*, *171*(4), 536–544. doi: 10.1086/528968

Yu, X., Lu, N., & Zhou, Z. (2008). Phagocytic receptor CED-1 initiates a signaling pathway for degrading engulfed apoptotic cells. *PLoS Biology*, *6*(3), 0581–0600. doi: 10.1371/journal.pbio.0060061

Yuan, S., Tao, X., Huang, S., Chen, S., & Xu, A. (2014). Comparative Immune Systems in Animals. *Annual Review of Animal Biosciences*, *2*, 235–258. doi: 10.1146/annurev-animal-031412-103634

Zanini, F., Anders, S., Pyl, P. T., & Pimanda, J. E. (2022). *Gene expression Analysing high-throughput sequencing data in Python with HTSeq 2 . 0*. *38*(March), 2943–2945.

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. doi: 10.1111/j.1749-6632.2009.04448.x

Zhang, Z., Strotz, L. C., Topper, T. P., Zhang, Z., Skovsted, C. B., & Brock, G. A. (2020). An encrusting kleptoparasite-host interaction from the early Cambrian. *Nature Communications*, *11*(2625), 1–7. doi: 10.1038/s41467-020-16332-3

Zhao, Jian-hua, & Guo, H. (2019). ScienceDirect Trans -kingdom RNA interactions drive the evolutionary arms race between hosts and pathogens. *Current Opinion in Genetics & Development*, *58–59*, 62–69. doi: 10.1016/j.gde.2019.07.019

Zhao, Junsong, Yao, Y., Li, D., Xu, H., Wu, J., Wen, A., … Ni, Q. (2018). Characterization of the Gut Microbiota in Six Geographical Populations of Chinese Rhesus Macaques (Macaca mulatta), Implying an Adaptation to High-Altitude Environment. *Microbial Ecology*, *76*, 565–577.

Zielen, S., Trischler, J., & Schubert, R. (2015). Lipopolysaccharide challenge: Immunological effects and safety in humans. *Expert Review of Clinical Immunology*, *11*(3), 409–418. doi: 10.1586/1744666X.2015.1012158

Zug, R., & Hammerstein, P. (2015). Wolbachia and the insect immune system: What reactive oxygen species can tell us about the mechanisms of Wolbachia-host interactions. *Frontiers in Microbiology*, *6*. doi: 10.3389/fmicb.2015.01201

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Academic training

03/12/2019: Good scientific practice, Prof. Dr. Martin Michel, Johannes Gutenberg University Mainz and Institute of Molecular Biology Mainz
23/01/2020 Convincing scientific presentations, Institute of Molecular Biology Mainz
02/03/2020: Introduction to R, Natasja Kreim, Institute of Molecular Biology Mainz
16/04/2020: GenEvo Advanced Series lecture, Various lecturers, Johannes Gutenberg University of Mainz and Institute of Molecular Biology Mainz
17/04/2020: Gene regulation, epigenetics and genome stability and associated topics, various lecturers, Institute of Molecular Biology Mainz
05/10/2020: RNA-seq analysis, Various instructors, Institute of Molecular Biology Mainz
19/10/2020: ChIP-seq analysis, Various instructors, Institute of Molecular Biology Mainz
03/11/2020 Introduction to Biostatistics, Fridolin Kielisch, Institute of Molecular Biology Mainz

04/03/2021 True Data – how to produce high quality research results, Martin Michels, Johannes Gutenberg University Mainz
19/05/2022 Scientific writing, Institute of Molecular Biology Mainz

Workshops

14/12/2020: Evolutionary design principles underlying gene regulation, Prof. Dr. Franz J. Weissing, Johannes Gutenberg University Mainz and University of Groningen
30/04/2021 Hypothesis Development and Experimental Design, Prof. Dr. Susanne Foitzik & Dr. Romain Libbrecht, Johannes Gutenberg University Mainz
13/12/2022 Research data management, Johannes Gutenberg University Mainz

Publications

- **Sistermans, T.**, Daras, H., Hartke, J., Stoldt, M., Libbrecht, R., Foitzik, S. (in prep.) Transcriptional networks indicate regulatory functions of parasite genes and the importance of infected worker prevalence for gene expression of host ants, uninfected nestmates and parasites.
- **Sistermans, T.**, Dirksen, P., Kaltenpoth, M., Foitzik, S. (in prep.) Tapeworm infection and a sham bacterial challenge result in independent changes in transcriptional activity and gut microbiome composition in a social insect.
- Gultyaev, A. P.; Koster, C.; Van Batenburg, D. C.; **Sistermans, T.**; Van Belle, N.; Vijfvinkel, D.; Roussis, A. (2023) Conserved structured domains in plant non-coding RNA enod40, their evolution and recruitment of sequences from transposable element. *NAR Genomics and Bioinformatics,* 16;5(4). doi: 10.1093/nargab/lqad091.
- **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(15), 4412–4426. doi: 10.1111/mec.16995
- 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*, 32:5877–5889. doi: 10.1111/mec.17155.
- Roelofs, D.; Zwaenepoel, A.; **Sistermans, T.**; Nap, J.; Kampfraath, A. A.; Van De Peer, Y.; Ellers, J.; Kraaijeveld, K. (2020). Multi-faceted analysis provides little evidence for recurrent whole-genome duplications during hexapod evolution. BMC Biology, 18(1). doi: 10.1186/s12915-020-00789-1

Student supervision

SS2020 Master Thies Abraham, Johannes Gutenberg University Mainz
SS2021 Bachelor Christine van Ooyen, The influence of an immune challenge in interaction with a cestode infection on survival, behaviour and the chemical profile. Johannes Gutenberg University Mainz
SS2022 Bachelor Anke Fost, Consequences of parasite load for the phenotype of *Temnothorax nylanderi* ants. Johannes Gutenberg University Mainz
WS2023 Master Nathalie Walther, Johannes Gutenberg University Mainz

Conferences

- 30 march – 1 April 2021. CSHL Biology and Genomics of Social Insects (virtual). Cold Springs Harbor, USA. Poster presentation: The dynamics of manipulation: how cestode parasites alter their host's phenotype

- 14 – 19 August 2022. ESEB Congress of the European Society of Evolutionary Biology. Prague, Czech Republic.
  Poster presentation: The effects of infection intensity on the gene expression on tapeworm infected ants
- 21 – 26 August 2022. ICOPA international congress of parasitology. Copenhagen, Denmark. Presentation title: Transcriptomic shifts with infection intensity in a system where parasitism extends host lifespan.

Competences

- **Laboratory skills**: qPCR, PCR, and Methylation Specific PCR (MSP), genetic cloning, DNA/RNA extraction, LPS injection of insects, microbial DNA extraction, dissection of ants, in-vitro cultivation of cestodes.
- **Bioinformatics skills**: worked with BLAST, BLASTp, BLAST2GO, the CODEML PAML package, Markov Clustering pipeline, MUSCLE alignment tool, PAL2NAL protein alignment, R, SPSS, Mfold, Needleman-Wunsch alignment and Clustal Omega alignment, done RNA-seq data analysis, GO enrichment analysis, KEGG pathway analysis, Reactome pathway analysis, amplicon sequencing analysis.
- **Fieldwork skills**: I've done fieldwork in both terrestrial and coastal ecology.
- **Others**: I've worked to reduce the environmental footprint of the lab I've worked in and hope to implement this in other labs.

Public and Scientific outreach

- 2013-2015: Writer for Media commission of student association *Gyrinus natans* at VU university Amsterdam
- 2014-2018: Student representative for information days/evenings in Biology bachelor/Ecology master at VU university Amsterdam
- 2019-2022: Elected PhD student representative of GenEvo steering committee
- 13/04/2021: Organizing seminar for Prof. Dr. Jacintha Ellers at Johannes Gutenberg University Mainz
- 2021-2023: Took leading role in reducing environmental footprint of lab, organized several talks and introduced several initiatives to reduce plastic, electricity usage and increase sustainability of scientific work
- 29/03/2023: Invited speaker at GreenYourLab Green Talk series (https://greenyourlab.org/). Presentation titled: Green Parasitology: Protecting parasites and fighting other parasites.

Spoken Languages

Dutch (native), English (C2), German (C1), Spanish (B2)

References

- Prof. Dr. Susanne Foitzik. Primary supervisor PhD.
  Email address: foitzik@uni-mainz.de
- Dr. Romain Libbrecht. Close collaborator during course of PhD.
  Email address: romain.libbrecht@univ-tours.fr
- Dr. James Weedon. Co-supervisor second master's thesis.
  Email address: james.weedon@vu.nl