Multidimensional effects of a manipulative helminth in its social host

DISSERTATION

Zur Erlangung des Grades Doktor der Naturwissenschaften

Am Fachbereich Biologie

Der Johannes Gutenberg-Universität Mainz

Vorgelegt von

Sara Beros
geb. am 07. 11. 1987
in Zenica, Bosnien & Herzegowina
Mainz, Januar 2019

Dekan: [removed for privacy purposes]

1. Berichterstatter: [removed for privacy purposes]
2. Berichterstatter: [removed for privacy purposes]

Tag der mündlichen Prüfung: [removed for privacy purposes]

Für mich.

"Look closely at nature. Every species is a masterpiece, exquisitely adapted to the particular environment in which it has survived."

Edward O. Wilson

"If I were asked to nominate my personal epitome of Darwinian adaptation, the ne plus ultra of natural selection in all its merciless glory… I think I'd finally come down on the side of a parasite manipulating the behaviour of its host – subverting it to the benefit of the parasite in ways that arouse admiration for the subtlety, and horror at the ruthlessness, in equal measure."

Richard Dawkins

Table of contents

Zusammenfassung

Sara Beros

Parasiten haben in Anpassung an ihre ausbeuterische Lebensweise faszinierende Ausbeutungstrategien entwickelt. Um die Übertragung von Wirt zu Wirt sicherzustellen, müssen gewisse Parasiten bestimmte Eigenschaften ihrer Wirte verändern. Beispielsweise werden Zwischenwirte häufig so verändert, dass sie Endwirten leichter zur Beute fallen. Das Phänomen der sogenannten Wirtsmanipulation ist auch in sozialen Insekten, u. a. in Ameisen, zu beobachten. Ameisen kommen weltweit vor und leben in hochkomplexen, sozialen Gemeinschaften, wodurch sie vielen, unterschiedlichen Parasiten optimale Bedingungen zum Überleben und zur Ausbreitung bieten. Dennoch besitzen auch soziale Insekten eine Reihe ausgeklügelter Verteidigungsstrategien, um sich und ihre Nestmitglieder zu schützen.

Diese Dissertation behandelt und fasst die phänotypischen Veränderungen zusammen, die in der Ameise *Temnothorax nylanderi* durch einen manipulativen Bandwurm ausgelöst werden. Der Bandwurm *Anomotaenia brevis* nutzt die Ameise als Zwischenwirt, kann sich aber nur in Vögeln sexuell fortpflanzen. Untersucht wurde wie sich das Verhalten, physiologische Eigenschaften, das Transkriptom, sowie der Lebenszyklus ändern. Neben infizierten Ameisen, sind die gleichen Aspekte auch in uninfizierten Nest- und Artgenossinnen untersucht wurden, um einen besseren Einblick über die indirekten Auswirkungen von Parasiten in sozialen Gruppen zu bekommen.

Meine Untersuchungen zeigen, dass infizierte Ameisen verstärkt inaktiv und in ihrem Fluchtverhalten beeinträchtigt sind, was sie zu einer leichten Beute für insektenfressende Vögel machen könnte (Kapitel 1 & 4). Die Infektion ist mit der Runterregulierung mehrerer Muskelgene assoziiert, wobei eine deutliche Muskelatrophie auf phänotypischer Ebene zu beobachten ist. Ebenfalls sind Gene, die mit Langlebigkeit in Zusammenhang gebracht werden, unterschiedlich exprimiert (Kapitel 3). Infizierte Arbeiterinnen werden nicht von ihren Nestgenossinnen verstoßen oder isoliert, sondern erhalten die beste Versorgung in der sozialen Gemeinschaft. Letzteres und die veränderte Expression potentieller Langlebigkeitsgene könnten die außergewöhnlich lange Lebensdauer von infizierten Arbeiterinnen erklären (Kapitel 1 & 5). Obwohl infizierte Ameisen sehr wahrscheinlich die ältesten Arbeiterinnen im Nest darstellen, zeigen sie auf physiologischer Ebene eine hohe Ähnlichkeit zu jungen Brutpflegerinnen: beide sind korpulent und fertil, haben einen ähnlichen Metabolismus und ähnliche chemische Erkennungsprofile (Kapitel 2; 4 & 5). Während infizierte Arbeiterinnen vom sozialen Engagement ihrer Schwestern scheinbar profitieren, haben letztere ein stark verkürztes Überleben (Kapitel 1 & 5). Zudem sind gesunde Nestgenossinen gegenüber Eindringlingen weniger aggressiv, was darauf hindeutet, dass parasitierte Kolonien in ihrer Verteidigung geschwächt sind (Kapitel 1).

Diese Arbeit deckt auf, dass der Bandwurm *A. brevis* das Verhaltensrepertoire seiner Wirtsameise reduziert und die Lebensdauer verlängert. Da das Überleben des Parasiten und dessen Übertragung zum Endwirt unweigerlich vom Überleben und Verhalten des Zwischenwirts, also der Ameise, abhängt, könnten einige Veränderungen adaptiv für den Parasiten sein. Zukünftige Studien sollten nun den kausalen Beweis liefern, dass der Parasit einen direkten Einfluss auf den Phänotypen der Ameise nimmt. Vorläufige Ergebnisse einer proteomischen Untersuchung zeigen, dass eine Vielzahl an parasitischen Proteinen in der Hämolymphe der Wirtsameise zirkulieren, und sehr wahrscheinlich am Manipulationsprozess beteiligt sind.

Summary

Sara Beros

Parasites have evolved fascinating exploitation strategies. Host manipulation refers to the ability of parasites to alter their host's phenotypes to their own advantage and is a widespread phenomenon of ecological and evolutionary importance. The success of trophically-transmitted parasites, which sequentially exploit multiple host species, largely depends on their ability to turn their hosts into easy prey. Parasites of social species exploit two hosts – the host organism which they directly infect, and the group to which the infected individual belongs. Social insects, in particular ants, are favourable hosts for various parasites including those capable of manipulation. While the insect's social lifestyle and high abundance offers parasites the possibility to establish and spread among group members, parasites also face a plethora of sophisticated defence strategies.

In this dissertation, I investigated the parasite-induced alterations on the individual and colony-level focussing on ants of *Temnothorax nylanderi*. Workers of this species are parasitically exploited as intermediate hosts by the trophically-transmitted tapeworm *Anomotaenia brevis*. I studied behavioural, physiological, life-history and transcriptomic changes in tapeworm-infected ant workers and their nestmates.

In detail, I found that tapeworm-infected ants are mainly inactive and impaired in their anti-predatory response (Chapter 1 & 4). Several muscle genes are downregulated upon infection and tapeworm-infected workers indeed suffer from muscle atrophy. Besides, multiple genes linked to longevity are differently expressed in tapeworm-infected workers (Chapter 3). Tapeworm-infected workers are tolerated by their nestmates and receive even more social care than the queen (Chapter 1 & 5). Intensive care combined with differently expression of longevity genes presumably enables tapeworm-infected workers to extend their lifespan (Chapter 1 & 5). In fact, I show that tapeworm-infected workers can live up to the old age of queens, which can live in this species up to two decades (Chapter 5). Surprisingly, old, tapeworm-infected workers resemble young nurses in many physiological aspects. Both are corpulent and fertile, and have similar metabolic rates and chemical profiles (Chapter 2; 4 & 5). While the increased social investment primarily benefits the infected individual, it comes at the cost to the colony. Uninfected nestmates invest a lot of time and energy into the well-being of their sick sisters, but live shorter lives than conspecifics from unparasitized colonies and are less aggressive towards enemies, which could potentially harm the entire group as their defence behaviours are reduced (Chapter 1 & 5).

In conclusion, I demonstrate that the tapeworm *A. brevis* reduces the behavioural repertoire of its individual host ant and increases its lifespan. I propose that these alterations possibly contribute to the tapeworm's survival and facilitate its transmission to definitive hosts, by taking advantage of the social lifestyle of its ant host. Future studies have to provide causative evidence that directly links the parasite to the observed changes. First indications suggest that *A. brevis* secretes proteins into its host haemolymph, of which one or more substances could be involved in the manipulation process.

General Introduction

Sara Beros

The evolution of parasitism and complex life cycles

Living at the expense of other organisms is the nature of parasites. Parasites use hosts as habitat, live in or on the hosts, deprive them from resources, and can cause severe damage, castration or even worse – death (Zelmer 1998). The parasitic lifestyle belongs to one of the most successful modes of life and has independently evolved in numerous lineages (Poulin & Morand 2000; Poulin & Randhawa 2015; Weinstein & Kuris 2016). Almost half of all described species are parasites and their diversity is tightly linked to the species richness of free-living organisms (Poulin & Morand 2000; Dobson et al. 2008; Kamiya et al. 2014; Poulin 2014). Parasites belong to a great diversity of taxa and come in all shapes and sizes: from viruses, bacteria, fungi and protozoan to species with more complex body structures such as helminths, arthropods or even birds as in the case of the charismatic avian brood parasites.

Parasites have developed various exploitation strategies, which have led, inter alia, to the adoption of complex life cycles (e.g. Parker et al. 2003; Poulin & Randhawa 2015). While parasites with simple life cycles complete their ontogeny in a single host (i.e. direct, one-host or homoxenous), parasites with complex life cycles rely on the sequential exploitation of multiple host species (i.e. indirect, multiple-host or heteroxenous), (e.g. Parker et al. 2015; Poulin & Randhawa 2015). Over the course of convergent evolution, complex life cycles have arisen repeatedly in distantly related parasites including some bacteria, many protozoans and most helminths (e.g. Auld & Tensley 2015; Poulin & Randhawa 2015). Specifically in the latter – with exception to monogeneans – all tapeworms, trematodes, acanthocephalans and many nematodes are parasites with complex life cycles depending on trophic transmission (Parker et al. 2015). Trophically-transmitted parasites require one or more intermediate hosts – often invertebrates – in which they start their life, grow and develop, and finally switch to a definitive host – often a vertebrate - to complete the development by reproducing sexually.

The incorporation of one or more hosts to the life cycle has provided unique fitness benefits (Choisy et al. 2003; Parker et al. 2003). Moving upwards the food chain, trophically-transmitted parasites eventually arrive in a larger, resource-richer and longer-living host that enables them to develop greater body size, longer lifespan and increased fecundity (Lafferty 1999; Parker et al. 2003). In addition, complex life cycles make it possible to reproduce with unrelated sex partners to circumvent inbreeding (Brown et al. 2001; Rauch et al. 2005). Yet, parasites with complex life cycles also face certain difficulties. They have to deal with multiple, different hostile enviroments, the hosts' physiology (Benesh et al. 2014). Eventually, each immune system might be more sophisticated than the previous one. Last but not least, parasites also encounter conspecifics or other parasite species with conflicting aims (Lafferty 1999; Haine et al. 2005;, Cézilly et al. 2014; Hafer & Milinski 2015a; 2016). Most importantly, however, parasites with complex life cycles need to ensure a successful switch to the correct next host. Transmission from one host species to another is an essential and critical step in the life of most parasites. The probability

to survive and to complete the life cycle is often reduced during transmission due to high parasite mortality (Dobson et al. 1992). Especially for trophically-transmitted parasites transmission constitutes a major challenge as the current host needs to be consumed by the next ranking one, generally conflicting with the survival interest of hosts. Accordingly, a successful transmission depends heavily on the probability of predation between host species.

The concept of host manipulation

Upon infection with a parasite, hosts frequently exhibit phenotypic alterations that appear to be beneficial to the parasite; either for its development, survival, transmission, dispersal or its offspring (e.g. Moore 2002; Hurd 2003; Libersat et al. 2009; Huhges et al. 2012). Despite changes in physiology and appearance, behavioural modifications are most prominent (Moore 2002). This very common phenomenon, observed in many hosts infected with parasites of diverse taxa and with different exploitation strategies, has been collectively referred to as *host manipulation* (Moore 2002; Hughes et al. 2012).

In the 20th century, biologist began to suspect that host modifications could be in the interest of the parasite. This hypothesis started to attract more attention since the early 1970's (e.g. Bethel & Holmes 1973), and finally research flourished when Dawkins introduced his conceptual work (1982), stating that host manipulation by parasites illustrates an example of the *extended phenotype*. Numerous studies demonstrated host modifications following parasite infection (Moore 2002), many times intuitively stating that changes are adaptive to the parasite without providing any or questionable experimental evidence (Cézilly et al. 2010; 2013; Poulin & Maure 2015). By the early 1990's, scientists viewed the manipulation hypothesis more critically (Moore & Goletti 1990; Poulin et al. 1994; Poulin 1995). To shed more light on the evolutionary reasons, Moore and Gotelli (1990) draw attention to the phylogenetic relationships among manipulative parasites and Robert Poulin emphasized that several criteria ("complexity", "purposiveness of design", "convergence" and "fitness effects") must be examined before regarding parasite-induced changes as true parasite adaptations (Poulin 1995). Despite considering the phenotypic alterations to be solely adaptive to the parasite, altered host phenotypes could be the result of host-mediated compensatory responses to limit the detrimental consequences of resource exploitation, and hence, adaptive to the host (Minchella 1985; Poulin et al. 1994; Lefèvre et al. 2008). Alternatively, parasite infection may induce physiological by-products and side-effects with no adaptive value, neither for parasite nor host (Poulin et al. 1994). Since then, more rigorous approaches have been made to study host manipulation (Thomas et al. 2005). Advances in genetics and molecular applications such as quantitative transcriptomics, proteomics and metabolomics as well as improved conceptual frameworks and computations tools have significantly contributed to our understanding of the underlying mechanisms of host manipulation (e.g. Biron et al. 2005; Hoover et al. 2011; Hughes 2013; Van Houte et al. 2013; Poulin & Maure 2015).

Host manipulation has been recognized as a widespread strategy employed by numerous parasite lineages (e.g viruses: Hoover et al. 2011; Van Houte et al. 2014; bacteria: Hussain et al. 2011; fungi: Maitland 1994; Hughes et al. 2011; helminths: Hurd et al. 2001; Seppälä et al. 2005; Franceschi et al. 2007; Benesh et al. 2008; Goodman & Johnson 2011; Wesołowska & Wesołowski 2014; protozoan: Berdoy et al. 2000; Poirotte et al. 2015; vector-borne parasites: e.g. Koella et al. 2002; Hurd 2003; Rogers & Bates 2007; Cornet et al. 2013; Smallegange et al. 2013; parasitoids: e.g. Eberhard 2000; Grosman et al. 2008; Libersat et al. 2009; Maure et al. 2011; social parasites: Jongepier et al. 2015; Elia et al. 2018). Trophically-transmitted parasites commonly induce changes in the activity, microhabitat choice and anti-predator behaviour of their intermediate hosts (e.g. Poulin 1994; Baldauf et al. 2007; Kaldonski et al. 2008; Médoc et al. 2009; Lafferty & Shaw 2013). They employ different manipulation strategies to optimise transmission (Hammerschmidt et al. 2009; Parker et al. 2009). Being transmitted too early or too late in the development may cause fitness costs for parasites. Hence, parasites protect their intermediate hosts by suppressing their susceptibility to predation during the early stage of infection, but turn their hosts into easier prey to facilitate transmission, once parasites become infectious for the next ranking host (e.g. Kaldonski et al. 2008; Dianne et al. 2011; Weinreich et al. 2013; Gopko et al. 2015). For instance, the amphipod *Gammarus pulex* serves as in intermediate host and hids more under refuges when its acanthocephalan parasite, *Pomphorphynchus laevis*, is in its non-infective stage (Dianne et al. 2011). As soon as the parasite becomes infective for several freshwater fishes, infected amphipods decrease their use of refuges (Kaldonski et al. 2008; Dianne et al. 2011). In addition, gammerids spend more time in areas with fish predator cues and also lose their attraction to conspecifics, which aggregate to diminish the risk of predation (Kaldonski et al. 2008; Durieux et al. 2012).

Eusociality and the life of social insects

In stark contrast to the parasitic way of life stands the evolution of group living and sociality in animals. Social life can be broadly characterised by the presence of communication, coordination and cooperation between the individuals of a group (Wilson 1971). The most advanced form of sociality – eusociality – marks a significant evolutionary achievement (Szathmáry & Smith 1995), but has only evolved in few orders of the animal kingdom (Wilson & Hölldobler 2005). The complex eusocial behaviours are best exemplified in the social insects, comprising all ants, some bees and wasps (Hymenoptera), and all termites (Isoptera). These insect societies are organised by reproductive and non-reproductive division of labour between highly related individuals of overlapping generations (Wilson 1971). The majority of female group members, the workers, have reduced their own reproductive potential to serve the interest of a few individuals that monopolise reproduction during their lifespan, the queens, and in termites also the king. All other colony-relevant chores are exclusively divided among the non-reproductive

workers, which specialize in one specific task such as brood care, food provisioning or nest defence (Wilson 1971).

Eusociality has truly led to the great ecological success of social insects, especially in ants (Wilson 1987). Yet, like any other lifestyle, it is also associated with costs. Insect societies offer optimal conditions for numerous parasites (Schmid-Hempel 1998; Quevillon & Hughes 2018). Social insects are ubiquitous across the terrestrial landscapes and their social life takes place in a colony; a socially stable environment comprising a high number of genetically similar individuals that constantly interact with each other. Parasites generally constitute a major selective force and shape the evolutionary ecology of their hosts (Schmid-Hempel 2011). The relevance of parasites for the social life of insect groups is reflected in their colony organisation (Naug & Smith 2007; Stroeymeyt et al. 2014; 2018) and their sophisticated defence mechanisms (Cremer et al. 2007). Beyond the individual-level defences that can be found in every solitary species, social insects have developed a suite of physiological and behavioural strategies that complement their collective defence repertoire (Cremer et al. 2007). In fact, some social insects have fewer immune genes than other insect species, which indicates that the additional defences are highly effective (e.g. Evans et al. 2006). The list of defences is long and diverse, and ranges from general responses such as avoiding parasites (e.g. Mehdiabadi & Gilbert 2002) or relocating nests in case of parasite contamination (e.g. Leclerc & Detrain 2018) to extremely sophisticated behaviours. The latter include for instance, the management of garbage and corpses (e.g. Waddington & Hughes 2010; Diez et al. 2012; 2014), collection of antimicrobial substances to disinfect the nest (e.g. Christe et al. 2003; Chapuisat et al. 2007), self-vaccination through the uptake of alive pathogen particles (e.g. Ugelvig & Cremer 2007; Konrad et al. 2012a), self-sacrifice of infected group members (e.g. Heinze & Walter 2010; Bos et al. 2012), living funeral of larger parasites inside the nest (e.g. Ellis et al. 2003; Greco et al. 2010), and the performance of social fevers of honey bees, whereby several bee workers simultaneously increase their body temperature to heat-kill fungi in their hive (Starks et al. 2000).

Host manipulation in social insects

There are numerous, striking examples of host manipulation, and some of the best-studied and most fascinating ones have been documented in social insects. Among the first and most famous cases of manipulative parasites is the trematode *Dicrocoelium dendriticum*, better known as the lancet liver fluke. *Dicrocoelium dendriticum* is a typical complex life cycle parasite relying on two intermediate hosts and several definitive hosts (i.e. preferably ruminants). Snails of *Zebrina* and *Cionella* species serve as first intermediate hosts and get infected by eating trematode eggs from faeces of definitive hosts (Krull & Mapes 1953). The parasitic eggs complete several developmental stages within the snail until being released over the snail's respiratory system covered in slime. Attracted by the slime balls are ant workers of *Formica* and *Camponotus* species, the second intermediate hosts (e.g. Krull & Mapes 1953; Hohorst & Graefe 1961; Carney 1969). Consumed parasitic

larvae migrate to different tissues within their ant host and develop to metacercariae, infectious to ruminants. While most metacercariae colonise the ant's haemocoel, a small number of metacercariae infest the host's head, with a single parasite that settles next to the suboesophageal ganglion inducing a temperature-sensitive behavioural alteration in the ant (Botnevik et al. 2016; Martín-Vega et al. 2018; **Figure 1A**). An infected ant worker leaves its colony to climb and to bite onto grass blades with its mandibles at early dawn, when temperatures drop. In this position, infected ants are presumably more susceptible to predation by grazing sheep and cows. Surviving ants return to their colony in the early morning, when temperatures start rising, only to repeat the aberrant behavioural procedure in the evening of the same day (Martín-Vega et al. 2018).

Another comparable example of behavioural manipulation involving the attachment onto vegetation is observed in 'zombie ants' (e.g. Hughes et al. 2011). Here, the parasites are not helminths, but parasitic fungi belonging to the species complex *Ophiocordyceps unilateralis* specialised in infecting ants of three genera of the Camponotini tribe (most investigations studying *Camponotus* ants), (e.g. Andersen et al. 2009; Hughes et al. 2011; Evans et al. 2011; de Bekker et al. 2014). Ant workers get infected during foraging trips by fungal spores attaching to the cuticle and penetrating into the ants' body cavity. The pathogen specifically infests the head of its host in high numbers (Hughes et al. 2011), secreting a set of metabolites among which two compounds - guanobutyric acid (GBA) and sphingosin – are known to be involved in neurological disorders and probably responsible for the behavioural alteration (de Bekker et al. 2014). Once infected, ants commit suicide in the interest of the parasite, demonstrating a stereotypical sequence of behaviours ceasing in the 'death grip' (Hughes et al. 2011). Similar to trematode-infected ants, a victim of *Ohiocordyceps* abandons its colony, climbs vegetation near the foraging trials and bites precisely into the major vein on the downside of a leaf, dying in this position. The ant's jaw remains locked due to atrophy of the mandibular muscles and prevents the ant from falling down when death occurs. Fungal hyphae start growing from the ant's corpse, forming a sexual structure for spore dispersal (e.g. Andersen et al. 2009; Hughes et al. 2011; **Figure 1B**). Manipulating the dead ant's position results in reduced fitness of the parasite, strongly implying that the fine-tuned behavioural alterations are adaptive to the parasite (Andersen et al. 2009).

In the next case, behavioural alterations are accompanied by changes in the physical appearance. *Myrmeconema neotropicum* is a parasitic nematode infecting ant workers of the tropical arboreal species *Cephalotes atratus* (Poinar & Yanoviak 2008). The nematode starts and completes its life cycle within a single host, the ant worker. Ants get infected as larvae when being fed with bird faeces containing nematode eggs. The parasitic eggs develop to juveniles and later to adults, and males and females copulate inside the ant's body cavity (Poinar & Yanoviak 2008). Until this stage, infected workers behave and look inconspicuously (Yanoviak et al. 2008). However, when fertilized female nematodes release hundreds of eggs inside the ant's abdomen, the exoskeleton in this body part gets stretched, which turns the black abdomen into a bright red one (Verble et al. 2012). In addition, workers are

changed in their behaviour. Ant workers become sluggish and show reduced defence responses (Yanoviak et al. 2008). Most interestingly, they start lifting their red abdomen while foraging, which probably makes them more vulnerable to predation by frugivorous birds, which mistake the red abdomen for juicy berries (Yanoviak et al. 2008; **Figure 1C**). In comparison to many other complex life cycle helminths depending on vertebrate hosts for their sexual reproduction, *Myrmeconema neotropicum* uses its bird host primarily for transport and distribution of its eggs to new locations, infecting new worker larvae of other *Cephalotes atratus* colonies (Yanoviak et al. 2008; Poinar 2012).

In this final example of host manipulation in social insects, the parasite is the strepsipteran *Xenos vesparum*, an obligate endoparasitoid, which induces a shift in the caste behaviour of its host, the paper wasp *Polistes dominulus* (Geffre et al. 2017). The life cycle starts with both sexes of *Xenos vesparum* infecting their single host by entering the wasp during its larval development via unmelanized openings of the cuticle (Beani 2006). Stylopized wasps (i.e. successfully infected by the strepsiteran insect) survive until adulthood, but exhibit an uncommon behaviour in relation to their social caste and life cycle. Stylopized females are typically workers, designated to perform colony-related tasks. Yet, they desert their colony shortly after emerging as callows and form extranidal aggregations with other stylopized non-nestmate conspecifics (Hughes et al. 2004). Nest desertion and aggregation are comprised in the behavioural repertoire of this species, but exclusively observed in gynes at the end of the reproductive phase, when females come together to collectively hibernate until next spring. A recent study reveals that gene expression patterns in the brains of stylopized females are shifted to gyne-related genes (Geffre et al. 2017). At the aggregation spots, male parasitoids pupate and disperse to mate with nearby females (Hughes et al. 2004; Beani et al. 2011). Female parasitoids remain inside their wasp host and only extrude their cephalothorax for mating (Hughes et al. 2004; Beani et al. 2011). Wasps infested by fertilized parasitoid females overwinter at the aggregations, often joined by unstylopized gynes. Next year in spring, when unstylopized gynes start founding their own colony, stylopized wasps begin to visit other nests and foraging sites, where parasitoid females preferably release their offspring to seek out new hosts (e.g. Beani & Massolo 2007; **Figure 1D**). It is likely that the behavioural alteration in stylopized females benefits the parasitoid insect as it facilitates mate location (Geffre et al. 2017). Moreover, stylopized females are sterilized by their parasitoid, unable to gain direct fitness, and initial wasp colonies may experience severe fitness costs due to the missing work force.

Figure 1. (**A**) 3D sagittal section of an infected ant worker harbouring a non-encysted metacercaria (nmc) of the trematode *Dicrocoelium dendriticum* in the suboesophageal ganglion in the brain (br) and multiple encysted metacercariae (emc) in the abdomen; © 2018, Martín-Vega. (**B**) A dead *Camponotus leonardi* ant displaying the characteristic "death grip" with a mature *Ophiocordyceps unilateralis* stroma arising from the ant's head; © 2011, David Hughes. (**C**) A *Cephalotes atratus* worker exposing her swollen, reddish abdomen in a conspicuous way when infected with the nematode *Myrmeconema neotropicum*, © 2008, Stephen P. Yanoviak. (**D**) Stylopized *Polistes*-female with four strepsipteran *Xenos vesparum* endoparasitoids lurking through the tergits of the wasp; © 2007, Werner Könecke.

Study system of this thesis

The research of this doctoral thesis focuses on the host-parasite interaction between the ant, *Temnothorax nylanderi*, and the trophically-transmitted tapeworm *Anomotaenia brevis* (**Figure 2**). The intermediate host, the ant *Temnothorax nylanderi,* lives in the deciduous forests of Western and Central Europe nesting in natural cavities on the forest floor (Heinze 1996; Foitzik & Heinze 1998; 2000). A colony consists of a single, singly-mated queen, her brood and workers, ranging from 10 to 300 adult ants, which are able to inhabit a single hollow acorn due to their small body size (e.g. Scharf et al. 2012a; Modlmeier et al. 2013). Other preferred nest sites are rotten pieces of wood, twigs or grass stems (e.g. Foitzik & Heinze 1998). Colonies of *Temnothorax* frequently relocate to new nest sites, fuse with other colonies for hibernation and display seasonal polydomy (Foitzik & Heinze 1998; Stroeymeyt et al. 2017). The latter means that one part of the colony splits up and occupies a nearby nest, but stays socially connected to the natal nest. Polydomy can be induced by high individual densities within a limited space and helps facilitating foraging performance (e.g. Foitzik & Heinze 1998; Cao 2013; Stroeymeyt et al. 2017). Workers forage solely, but recruit nestmates via 'tandem runs' when oversized food items are discovered (Heinze et al. 1996; Glaser & Grüter 2018). *Temnothorax* ants feed on dead and living arthropods, plant material and on secretions and excretions of other animals (Stroeymeyt et al. 2017). The latter can sometimes be contaminated with parasite propagules (e.g. Plateaux 1972; Carney 1969; Heinze et al. 1998; Yanoviak et al. 2008). Foragers of *T. nylanderi* come across bird droppings containing tapeworm proglottids filled with eggs of *Anomotaenia brevis* (e.g. Plateaux 1972; Buschinger 1973; Gabrion et al. 1976; Trabalon et al. 2000). The adult tapeworm lives and sexually reproduces in the intestines of several closely related bird species (e.g. Plateaux 1972; Buschinger 1973; Gabrion et al. 1976; Trabalon et al. 2000). Tapeworm eggs are released with the bird's faeces and picked up by foraging ants. Brought back to the ant colony, tapeworm eggs are fed to colony members. The parasitic oncospheres hatch after ingestion, penetrate the gut wall and enter the ant's haemocoel, where they develop into larvae called cysticercoids. These cysticercoids are infectious for the definitive bird host and linger in the ant's body until being transmitted through predation to complete the life cycle (**Figure 2**).

All ant castes can get infected, but mostly workers are (Scharf et al. 2012b). When worker larvae are fed with tapeworm eggs, they display a strong aberrant physical appearance as adults. Unlike their brownish nestmates with a dark abdominal strip, tapeworm-infected workers are completely yellow, smaller and they present an altered chemical signature on their softer cuticle (Trabalon et al. 2000; Scharf et al. 2012b). The lighter pigmentation of ants is a common modification when infected with endoparasites (e.g. Muir 1954; Crosland 1988; Carney 1969; Heinze et al. 1998; Yanoviak et al. 2008). In addition to the severe changes in appearance and physiology, infection induces behavioural alterations. Tapeworm-infected workers linger most of their time on the brood in close proximity to other tapeworm-infected

workers, the queen and uninfected nurses (Scharf et al. 2012b). Also tapeworm-infected virgin queens remain inside their natal colony, probably unable to successfully start a colony on their own (S. Beros and S. Foitzik, pers. communication). The described host modifications can be induced by a single cysticercoid of *A. brevis*, but a single ant can carry several dozen of cysticercoids (highest record: 78; S. Beros, pers. observation); (Scharf et al. 2012b). Infections with *A. brevis* have been also confirmed in adult ants displaying the normal brown phenotype (Scharf et al. 2012b). These workers possibly infect themselves as adults by ingesting tapeworm eggs, either through consumption of contaminated food or via trophallaxis. It is unlikely that the parasitic larvae are transmitted by tapeworm-infected workers. Firstly, cysticercoids reside in the ant's body cavity. Secondly, new occurring infections were never found in long-term observations of colonies in the laboratory (S. Beros, pers. observation). Social insects respond to infected group members and adjust their behaviour accordingly (e.g. Ugelvig & Cremer 2007; Richard et al. 2008; Walker & Hughes 2009; Baracchi et al. 2012). Workers in parasitized ant colonies do not isolate or avoid tapeworm-infected nestmates, but rather care for them and show intermediate activity levels compared to conspecifics from unparasitized colonies (Scharf et al. 2012b). Important fitness correlates such as *per-capita* productivity remains unaffected (Scharf et al. 2012b). Yet, parasitized *T. nylanderi* colonies shift their investment strategies (Scharf et al. 2012b), producing more and heavier males, and more inter-caste individuals (Okada et al. 2013). These findings suggest that the extensive phenotypic alterations on the individual level are buffered by the society and do not scale up to the colony level (Scharf et al. 2012b).

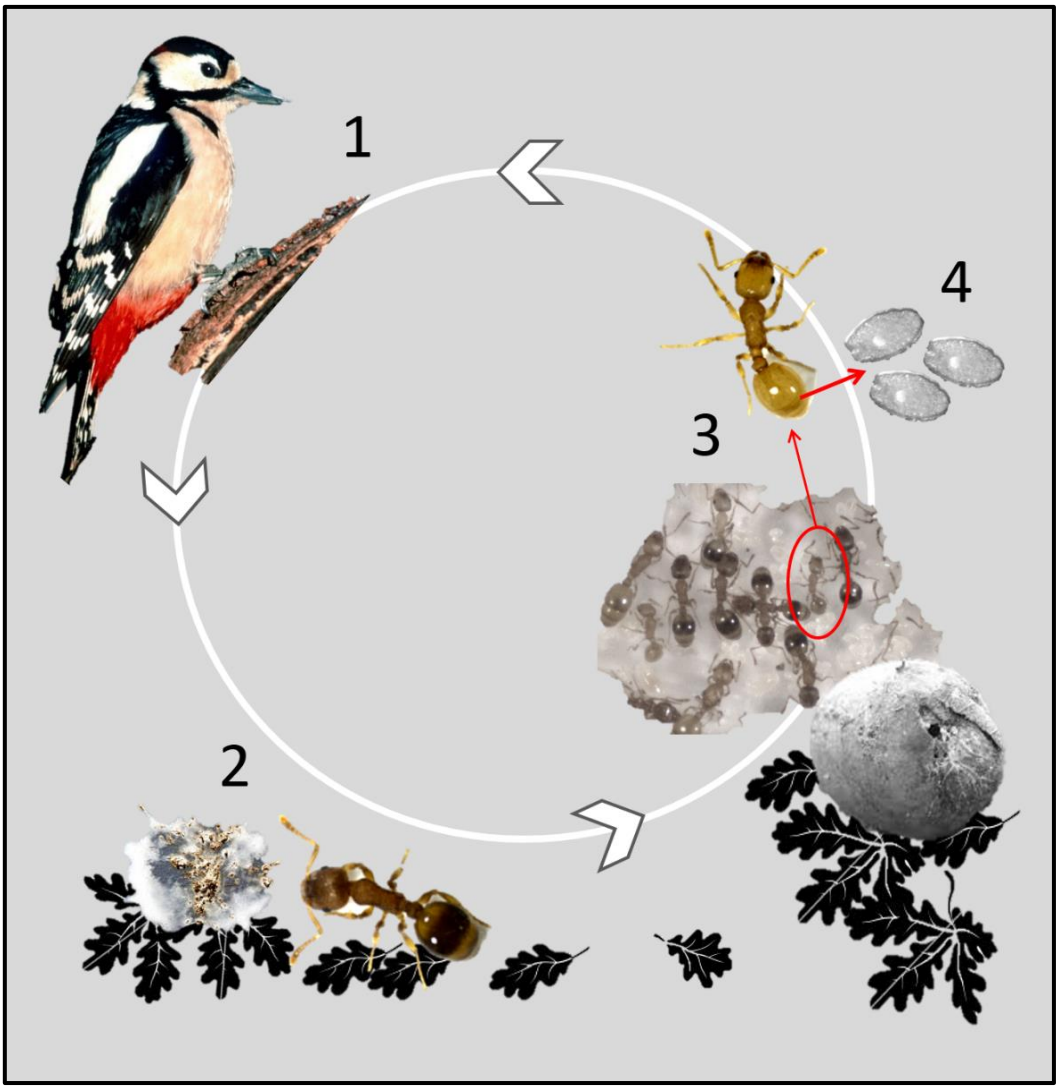

Figure 2. The life cycle of *Anomotaenia brevis*. Adult tapeworms live in the digestive system of their definitive bird hosts, different woodpecker species (1). Tapeworm eggs are released with bird droppings, left on leaves or branches on the forest floor, and get picked up by foraging ant workers of *Temnothorax nylanderi* (2). Foragers return to their colony and feed their infant sisters (ant larvae) with contaminated bird faeces, a high protein food source. The infection causes a smaller body size and changes the body colour from brown to yellow (3). Tapeworm eggs develop inside the ant's abdomen to larvae (cysticercoids), infectious for the definitive hosts (4). *Anomotaenia brevis* completes its life cycle when its intermediate ant host is consumed by its definitive bird host.

Aims of this thesis

It is well known that manipulative parasites generally induce changes in multiple traits (e.g. Thomas et al. 2010; Cézilly et al. 2013). Yet, the majority of studies have concentrated on a single phenotypic trait. Because behaviour has a great impact on the survival and fitness of an animal, the focus of most investigations has been

placed on behavioural alterations due to parasitism by manipulative parasites. However, besides behaviour also other phenotypic traits are commonly altered and especially life-history components are of great importance (e.g. Minchella 1995; Agnew et al. 2000). In tapeworm-infected *T. nylanderi* workers, parasite-induced alterations have been documented for some behavioural, morphological and physiological traits (Trabalon et al. 2000; Scharf et al. 2012b). However, at the onset of this thesis, the known alterations did not help to explain how the tapeworm could benefit from it. Although phenomenological work is crucial for our understanding of host manipulation, research has to include investigations of the proximate mechanisms (Hughes 2013; Herbison et al. 2018; Hughes & Libersat 2018). In order to elucidate the pathways potentially exploited by parasites, a better insight can be gained by studying the alterations on the molecular level.

Unlike solitary species, social species offer parasites two hosts. Parasites of social insects infected two hosts at once, because the parasite's direct host is always part of a cohesive society (Schmid-Hempel 1998; Hughes et al. 2012). Although social insects are frequently exploited by manipulative parasites, most research has focused on the single, infected social insect. One reason is that infected ant, bee and wasp workers exhibit very unusual behaviours, such as they often abandon their colony without ever returning (e.g. Carney 1969; Yanoviak et al. 2008; Hughes et al. 2011). This makes it difficult, of course, to study how nestmates behave towards infected group members and *vice versa*, and we thus have a poor understanding of the potential colony-level effects. My study system offers the unique opportunity to study the effects of manipulative parasites beyond the single, infected host, because tapeworm-infected workers rarely leave the nest and engage in social interactions with their uninfected nestmates (Scharf et al. 2012b).

The research questions in this thesis are diverse and each chapter covers several research interests. The overarching aim, however, was to extend our knowledge of parasite-induced modifications on the individual and colony-level in social hosts (chapter 1 – 5), and to gain a better understanding of the molecular alterations that shape the host's phenotype (chapter 3). Across all five chapters, I always also addressed the question how uninfected nestmates in parasitized colonies are affected by the presence of their tapeworm-infected sisters and how they differ from ants of unparasitized colonies. In chapter 1, I studied the survival of different worker types (i.e. infected/ uninfected), their anti-predatory behaviour and the colonies' aggression behaviour. In chapter 2, I more closely investigated the underlying mechanisms of nestmate recognition, as I found that aggression declines with parasitsm. In chapter 3, I focused on the molecular basis of host manipulation and specifically contrasted the gene expression patterns of different worker types. Finally, in chapter 5, I studied the lifespan of *T. nylanderi* ants over three consecutive years and additionally examined a battery of different physiological parameters and the investment in social care towards tapeworm-infected workers.

Chapter 1

The parasite's long arm: a tapeworm parasite induces behavioural changes in uninfected group members of its social host

Sara Beros, Evelien Jongepier, Felizitas Hagemeier and Susanne Foitzik

Based on:
Proceedings of the Royal Society of London B: Biological Sciences, 282, 20151473

Abstract

Parasites can induce alterations in host phenotypes in order to enhance their own survival and transmission. Parasites of social insects might not only benefit from altering their individual hosts, but also from inducing changes in uninfected group members. *Temnothorax nylanderi* ant workers infected with the tapeworm *Anomotaenia brevis* are known to be chemically distinct from nestmates and do not contribute to colony fitness, but are tolerated in their colonies and well cared for. Here, we investigated how tapeworm-infected workers affect colony aggression by manipulating their presence in ant colonies and analysing whether their absence or presence resulted in behavioural alterations in their nestmates. We report a parasite-induced shift in colony aggression, shown by lower aggression of uninfected nestmates from parasitized colonies towards conspecifics, potentially explaining the tolerance towards infected ants. We also demonstrate that tapeworm-infected workers showed a reduced flight response and higher survival, while their presence caused a decrease in survival of uninfected nestmates. This anomalous behaviour of infected ants, coupled with their increased survival, could facilitate the parasites' transmission to its definitive hosts, woodpeckers. We conclude that parasites exploiting individuals that are part of a society not only induce phenotypic changes within their individual hosts, but in uninfected group members as well.

Keywords: parasite-induced alterations, extended phenotype, survival, aggression, recognition, social insects

Introduction

Parasites have developed a number of fascinating strategies to infect, thrive and reproduce within their hosts (Moore 2002). Among these strategies is the manipulation of host appearance and behaviour, where the altered host traits may be regarded as the parasites' *extended phenotype* (Dawkins 1982). Host manipulation is frequently observed in parasites possessing complex life cycles reliant upon trophic transmission (Moore 2002). The most severe parasite-induced alterations are exhibited by intermediate hosts, whose aberrant phenotypes can contribute to parasites' transmission into subsequent hosts, furthering the parasites' life cycle (Poulin 2000; Poulin 2010; Thomas et al. 2005; Cézilly et al. 2010). Many manipulative parasites use group-living organisms such as social insects as hosts (Schmid-Hempel 1998). Indeed, some of the most prominent examples of host manipulation have been observed in social insects infected with endoparasites. For instance, *Formica* and *Camponotus* ants infected with the lancet liver fluke attach themselves to the tips of grass blades, where they are more likely to be ingested by grazing mammals, the parasite's definitive hosts (Carney 1969). Intermediate ant hosts infected with the nematode *Myrmeconema neotropicum* linger in rainforest canopies, lifting their red, berry-like abdomen to draw the attention of nearby avian hosts (Yanoviak et al. 2008).

While our understanding of such parasite-induced alterations in individually infected hosts is rapidly growing (e.g. proximate mechanisms (Adamo 2013)), it is less well understood how the altered phenotypes of infected hosts affect other social group members. For instance, parasite infections in social insects change their hosts' chemical signature (e.g. Salvy et al. 2001; Dapporto et al. 2007; Richard et al. 2008; McDonnell et al. 2013), which may in turn affect social communication, and thus the functioning of societies. Such parasite-induced changes in cuticular hydrocarbon profiles (Salvy et al. 2001; Dapporto et al. 2007; Richard et al. 2008; McDonnell et al. 2013) may enable group members to recognise infected individuals, and to either provide enhanced care towards infected members to reduce the risk of pathogen spread (Aubert & Richard 2008; Walker & Hughes 2009) or to expel them from the colony if their maintenance is costly (Ibrahim & Spivak 2006; Baracchi et al. 2012). Social insects use cuticular hydrocarbons generally as recognition cues to discriminate nestmates from non-nestmates (Martin et al. 2008; Dani et al. 2005). They detect differences based on the quantitative variations of cuticular hydrocarbons (Cini et al. 2009). Usually, individuals within a colony share a colony-specific odour, which results from the frequent exchange of recognition cues among group members (reviewed in (Van Zweden & D'Ettorre 2010)). The presence of infected individuals with altered chemical profiles may lead to a more diverse chemical signature within colonies, increasing the likelihood of odour overlap with other conspecific colonies, which share the same cuticular hydrocarbons but in different quantities (reviewed in (Van Zweden & D'Ettorre 2010)). Consequently, group members of infected workers may be impaired in their nestmate recognition, thus affecting colonies' defence against intruders.

An interesting model to investigate parasite-induced changes on the individual and collective level is the interaction between the common endoparasitic tapeworm *Anomotaenia brevis* and its intermediate host, the ant *Temnothorax nylanderi* (formerly *Leptothorax nylanderi* [Plateaux 1972; Trabalon et al. 2000; Bolton 2003]). Ants become infected as larvae, after they are fed with bird faeces containing tapeworm eggs (Trabalon et al. 2000). The parasitic tapeworm penetrates the ant's gut wall and transforms into a cysticercoid within the haemocoel. In this stage, the parasite cannot be transmitted between adult ants. It completes its life cycle when ants are preyed upon by the definitive hosts, woodpeckers (Trabalon et al. 2000). Most tapeworm-infected ants are easily identified by their smaller size and their yellow cuticle (**Figure 3**), which contrasts with the regular brown coloration of *T. nylanderi* ants. Moreover, they exhibit a quantitatively different cuticular hydrocarbon profile (Trabalon et al. 2000), which may affect their group members' nestmate recognition. Workers can be infected with several cysticercoids, and chemical as well as morphological alterations become more pronounced with increasing parasite load (Trabalon et al. 2000; Scharf et al. 2012b). On the behavioural level, infected workers are inactive and remain inside the nest, where they rarely engage in social tasks such as brood care (Scharf et al. 2012b).

The aims of this study were threefold. First, by simulating nest attacks, we tested whether infected workers were less likely to evacuate their nest than uninfected ants. Infected workers that remain inside their nest when attacked are likely to be eaten by woodpeckers. The potential lack of a flight response is thus thought to promote the transmission of the parasite to its definitive host. Second, we investigated whether worker survival is related either to the infection status (i.e. infected with *A. brevis* or not) or the presence of infected workers within a colony. A previous study showed that uninfected workers provide better care for infected than for uninfected nestmates (Scharf et al. 2012b), which may come at the expense of their own survival. As infected workers are tolerated in their colony and well cared for (Scharf et al. 2012b), we finally investigated whether colony aggression, an important collective trait involved in nestmate recognition and nest defence, is affected by the presence of infected workers. Specifically, we evaluated the aggressive responses of ant colonies that were either naturally parasitized (i.e. with infected workers) or unparasitized (i.e. without infected workers). We then repeated the aggression tests following experimental manipulation of colony parasitism status (i.e. the absence or the presence of infected workers). If infected workers may lead to a broader colony odour, we predicted parasitized colonies to be less aggressive, in particular towards conspecifics.

Methods

Ant collection and maintenance

From April to June 2013, we collected 221 ant colonies in an oak forest near Mainz, Germany (50°00′35.7″ N, 8°10′48.6″ E). Colonies of *T. nylanderi* consist of several dozen monomorphic workers and a single queen, which reside in preformed natural cavities (e.g. acorns, sticks) in the leaf litter of deciduous forests throughout Europe (e.g. Heinze et al. 1996). Parasite pressure in the local population is high, as approximately 30% of the colonies contain one or more tapeworm-infected individuals (Scharf et al. 2012b). Ant colonies were transferred in their natural nest structures into ziploc bags, transported to the laboratory and relocated to artificial observation nests composed of a piece of Plexiglas with a pre-cut rectangular cavity (50 × 10 × 3 mm; 3 mm wide entrance) sandwiched between two microscope glass slides. These nests were placed in 100 × 100 × 30 mm boxes with a plaster floor. Colonies were fed weekly with honey, crickets and water. Unless otherwise stated, colonies were kept at 18°C : 14°C in a 12 L : 12 D cycle.

Parasitism by Anomotaenia brevis

After collection, we counted the number of brood and adult ants per colony, differentiating between workers of the common brown and yellow phenotype. Parasitized colonies from this collection (n = 59, 26.7%) harboured 1–32 infected workers, yielding intra-colonial parasitism rates of 2–54% (15.1 ± 11.9%, mean ± s.d.). In a previous study (Scharf et al. 2012b), we confirmed that workers of the yellow phenotype are invariably infected with tapeworm cysticercoids; however, 2% of the workers exhibiting the brown phenotype in parasitized colonies can carry *A. brevis* cysticercoids as well.

Nest disturbance

To assess whether infected workers were indeed less likely to escape following nest disturbance, potentially promoting predation of the parasite by the definitive host, we cracked the surface of 40 undamaged acorns containing *T. nylanderi* colonies and shook them for 10 s. We recorded the number of infected and uninfected workers observed escaping the nest within a 90 s timeframe and calculated the proportion of escaped workers based on the colony counts obtained the next day. Of these 40 colonies, 16 contained 1–32 infected ants. Fourteen colonies (six parasitized and eight unparasitized) were later used in the aggression experiments.

Experimental design

For the aggression experiments, we selected 114 colonies, 38 of which contained workers infected with *A. brevis* (range: 1–27 infected workers; parasitism rate: 15.4 ± 10.3%, mean ± s.d.). As the presence of the queen can influence workers' aggression (Stroeymeyt et al. 2007), we chose only colonies with a resident queen. Colony sizes (range: 20–140 workers; 58.3 ± 28.2, mean ± s.d.) did not differ between parasitized and unparasitized colonies (Wilcoxon test: W = 22219, p = 0.920). The effect of infected nestmates on colony aggression was assessed before and after manipulation of the colonies' parasitism status. We manipulated the parasitism status of colonies by adding or removing workers using a blocked experimental design (**Figure 3**). We first allocated colonies into one of 19 blocks, with each block consisting of two parasitized and four unparasitized colonies that were subjected to the aggression tests on the same day. Colonies assigned to the same block were comparable in worker number, and the two parasitized colonies exhibited similar parasitism rates. Within blocks, unparasitized and parasitized colonies were assigned to six treatments. Treatments included (I) parasitized–control, (II) unparasitized–control, (III) parasitized–unparasitized by removing infected workers and (IV) unparasitized–parasitized by adding infected workers. To control for the removal or addition of workers, we further included (V) unparasitized–unparasitized by removing uninfected workers and (VI) unparasitized–unparasitized by adding uninfected workers.

The manipulation of colony composition is possible in *T. nylanderi* as colony take-over and merging occur commonly in the field (Foitzik & Heinze 1998). Nonetheless, steps were taken to reduce the likelihood of rejection of transferred non-nestmates: workers and their recipient colony were anaesthetized with CO_2; nests were gently shaken after worker addition to promote the passive exchange of cuticular hydrocarbons; and colonies were cooled to 8°C for one week during which expelled workers were reintroduced. Control colonies (treatments I and II) and colonies from which workers were removed (treatments III and V) were likewise subjected to anaesthetization and cooling. Manipulation failed in three colonies receiving infected workers as all transferred workers were found dead. These were excluded from further analyses. Because we could not determine worker acceptance in colonies that received (indistinguishable) uninfected workers, we repeated the addition of infected (n = 20) and uninfected workers (n = 20) with 40 other colonies using metal-wire-marked ants. These experiments showed that 76.88% of the infected and 60.38% of the uninfected workers were accepted. Hence, infected workers tended to be more - though not significantly - readily accepted (quasi-binomial generalized linear model: F = 2.77, Δ d.f. = 1, p = 0.108).

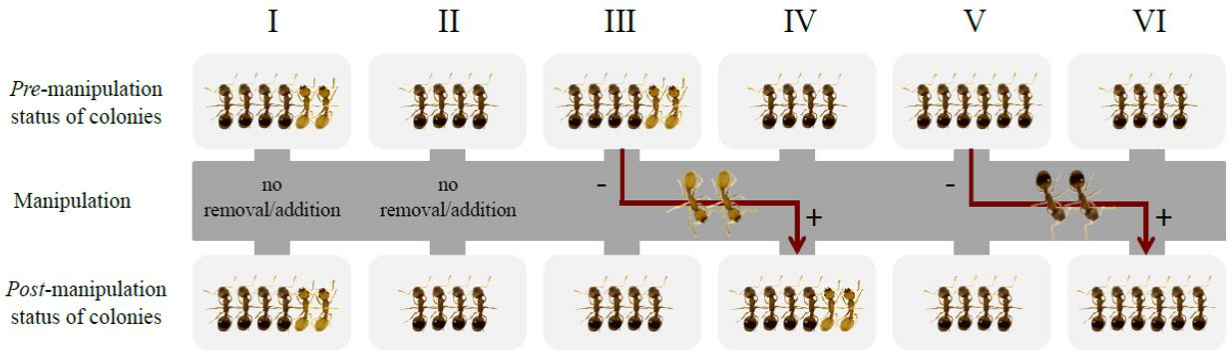

Figure 3. Experimental design: 114 *T. nylanderi* colonies were allocated to 19 blocks. Each block consisted of six colonies, which were assigned to six different treatments (I–VI). Colonies belonging to treatments I and II remained unchanged. Infected workers from naturally parasitized colonies assigned to treatment III were removed and added to naturally unparasitized colonies of treatment IV. Naturally unparasitized colonies of treatments V and VI served as controls for the numerical manipulation of workers. The numbers of removed and added workers (i.e. infected and uninfected) within one block were identical.

Behavioural experiments: colony-level aggression

Pre-manipulation aggression was assessed 17.8 ± 11.9 (mean ± s.d.) days after collection and post-manipulation aggression 30 days after manipulation. Colony aggression in this species is consistent (Scharf et al. 2012a), thus we expected any change in aggression to be the result of manipulating the parasitism status of the colony. Every colony was confronted with four non-nestmate opponents from the same study site: (i) an infected worker from a parasitized *T. nylanderi* colony; (ii) an uninfected worker from a parasitized *T. nylanderi* colony; (iii) an uninfected worker from an unparasitized *T. nylanderi* colony; and (iv) an uninfected *T. affinis* worker. Ants often show higher aggression towards ants of a different, but related species (Scharf et al. 2011) as they are more easily recognized as alien by their qualitatively different chemical profile. To exclude variance generated by the opponents' behaviour (Roulston et al. 2003), and because aggression against live and dead opponents is correlated in *Temnothorax* ants (Scharf et al. 2012a; Modlmeier & Foitzik 2011), we used a single, freshly defrosted opponent for each test. The opponent was carefully positioned inside the nest, next to the ant cluster and the nest entrance was sealed. For 5 min, we recorded the behaviour of each ant interacting with the opponent at 20–30 s intervals (i.e. within the first minute every 20 s, after that every 30 s), yielding 11 observations per aggression test per colony.

We differentiated between ants that behaved aggressively (i.e. mandible spreading, dragging, holding, biting and very rarely stinging) or non-aggressively (i.e. grooming and antennating), as well as between uninfected and infected workers (i.e. in parasitized colonies) interacting with the opponent. We calculated the proportion of aggressive interactions based on the total number of aggressive and non-aggressive

interactions for each test. Tests against the four opponents were carried out consecutively over 4 days (with a 24 h time interval between tests) in a randomized order to control for potential carry-over effects. Experiments were conducted blindly where possible (note that infected workers were apparent due to their yellow coloration). Nests were reopened following each aggression test, and opponents were removed and subsequently inspected for cysticercoids. Dissections confirmed that all opponents of the yellow phenotype contained cysticercoids (min.–max.: 1–56). Only 1.6% of the 684 opponents of the regular phenotype (i.e. brownish coloured *T. nylanderi*, $n = 9$; reddish coloured *T. affinis*, $n = 2$) carried cysticercoids. The associated aggression tests were excluded from further analyses.

Worker survival

The consequences of tapeworm infection on worker survival were assessed by counting the workers on the day of manipulation and then by recounting them when the first new workers began to eclose from the pupae. We only included naturally parasitized and unparasitized colonies (i.e. treatments I and II), because these had suffered from parasitism for a longer time span. Although counting times varied (103 ± 32 days, mean ± s.d.) due to differences in brood development between colonies, time intervals did not differ between parasitized and unparasitized colonies (Wilcoxon test: $W = 170.5$, $p = 0.780$, $n_{\text{colonies}} = 38$). Moreover, the survival of infected and uninfected workers from the same colony was assessed on the same day.

Statistical analyses

To assess whether or not worker types (i.e. infected and uninfected workers from parasitized colonies, uninfected workers from unparasitized colonies) differed in their response to nest disturbance, we used a generalized linear mixed model (GLMM) with binomial distribution and logit-link function. The proportion of workers that evacuated their nest upon disturbance (i.e. number of evacuating versus non-evacuating workers) was fitted as the dependent variable. The worker type was included as the fixed predictor and colony identity served as a random factor. We used penalized quasi-likelihood parameter estimation to account for overdispersion (i.e. glmmPQL function implemented in the MASS package (Venables & Ripley 2002)). To evaluate whether natural or experimental parasitism by *A. brevis* affected the aggressive responses of colonies, we used quasi-binomial GLMMs (glmmPQL) with logit-link function. The proportion of aggressive interactions (i.e. aggressive versus non-aggressive) served as the dependent variable in separate analyses of pre- and post-manipulation aggression. For the analyses of pre-manipulation aggression, we fitted the natural parasitism status of colonies, the opponent type and their interaction as fixed predictors. The time interval between collection and the first aggression trial as well as the colony size served as covariates in the initial model, but only the former was retained in our final model (time interval: Wald, $\chi^2_1 = 6.30$, $p = 0.012$; colony size: Wald, $\chi^2_1 = 0.44$, $p = 0.510$). For the analyses of post-manipulation aggression, we included the natural and current

parasitism status, the opponent type and their interactions as fixed predictors. The type of manipulation (i.e. worker removal/addition/no numerical change) was fitted as a cofactor to account for the change in colony size due to the manipulation. Additionally, we evaluated the association between the aggression towards infected opponents and their parasite load (i.e. number of cysticercoids of *A. brevis*). Here, the proportion of aggressive interactions towards infected opponents was used as the dependent variable, the number of cysticercoids as a continuous variable and the time interval between collection and testing as a covariate. In all three analyses of colony aggression, colony identity nested in block identity was entered as a random factor. For model selection, we used a backwards-stepwise procedure (α = 0.05) based on the Wald χ^2-test (Lesnoff & Lancelot 2012). To rule out that differences in the aggressive responses between parasitized and unparasitized colonies were due to the lethargic behaviour of infected workers, we repeated the analyses excluding the interactions of infected workers. We only report the latter results, as infected workers performed only 1.75% of all observed interactions, and their exclusion did not yield qualitatively different outcomes. To assess the differences in worker survival, we used a binomial GLMM with logit-link function (Bates et al. 2012), fitting the proportion of workers that survived (i.e. surviving versus non-surviving workers) as the dependent variable, worker type as the fixed predictor and colony identity as a random factor. All statistical analyses were performed in R v. 2.15.2 (R Core Team 2012).

Results

Nest disturbance

Worker types (i.e. infected or uninfected) differed in their response to nest disturbance (GLMM: Wald, χ^2_2 = 17.89, p < 0.001). Only five out of 155 infected (3.2%) ants escaped, whereas 634 out of the 1075 uninfected workers (58.9%) from parasitized colonies and 677 out of the 1410 workers (48.0%) from unparasitized colonies fled. Thus, infected workers less frequently evacuated their nests compared with uninfected workers from parasitized (t_{15} = −4.08, p = 0.001) or unparasitized colonies (t_{14} = −3.34, p = 0.005). There was no difference between the latter two worker types (t_{14} = 0.82, p = 0.425).

Aggression before manipulation

Naturally parasitized colonies were less aggressive than unparasitized colonies (GLMM: Wald, χ^2_1 = 9.80, p = 0.002; **Figure 4*a***), although this effect also depended on the opponent type (parasitism status × opponent type: Wald, χ^2_3 = 9.70, p = 0.021; **Figure 4*b***). Parasitized colonies were less aggressive towards non-nestmate conspecifics than unparasitized colonies, regardless of whether the opponent was an infected worker (t_{92} = −2.90, p = 0.005), an uninfected worker from a parasitized colony (t_{92} = −2.96, p = 0.004) or an uninfected worker from an unparasitized colony (t_{92} = −2.77, p = 0.007; **Figure 4*b***). Conversely, parasitized and unparasitized colonies

responded with similar high aggression towards *T. affinis* workers (t_{92} = 0.05, p = 0.963). Colonies were more aggressive towards infected than towards uninfected opponents (from parasitized colonies: t_{328} = 1.94, p = 0.053; from unparasitized colonies: t_{328} = 2.48, p = 0.014), although this higher aggression was independent of the parasite load (i.e. the number of cysticercoids within infected ants; Wald, χ^2_1 = 0.02, p = 0.877). There was no difference in aggression towards uninfected workers from parasitized and unparasitized colonies (t_{328} = 0.55, p = 0.583). Colonies responded most aggressively towards *T. affinis* workers compared with infected *T. nylanderi* workers (t_{328} = 5.48, p < 0.0001), uninfected *T. nylanderi* workers from parasitized colonies (t_{328} = 7.21, p < 0.0001) and *T. nylanderi* workers from unparasitized colonies (t_{328} = 7.64, p < 0.0001).

Aggression after manipulation

Post-manipulation aggression was lower in currently parasitized (treatments I and IV) than in unparasitized colonies (treatments II, III, V and VI; GLMM: current parasitism status: Wald, χ^2_1 = 10.40, p = 0.001; **Figure 4*a***). Colony aggression varied with the opponent type (Wald, χ^2_3 = 12.30, p = 0.006), but there was no interaction between current parasitism status and opponent type (Wald, χ^2_3 = 3.56, p = 0.313). Colonies were more aggressive towards heterospecific opponents than towards infected workers (t_{319} = −3.38, p < 0.001), uninfected workers from parasitized colonies (t_{319} = −1.91, p = 0.057) and uninfected workers from unparasitized colonies (t_{319} = −2.52, p = 0.012). Post-manipulation aggression did not differ between the three conspecific opponents (all p > 0.1). Post-manipulation aggression was unrelated to the natural parasitism status of colonies (Wald, χ^2_3 = 1.78, p = 0.182) or its interaction with experimental parasitism status (Wald, χ^2_3 = 0.25, p = 0.616), opponent type (Wald, χ^2_2 = 6.55, p = 0.088) or both (Wald, χ^2_3 = 7.23, p = 0.065). Hence, the effect of the current parasitism status (treatments I and IV) on post-manipulation aggression did not differ between manipulated (treatments III and IV) and control colonies (treatments I, II, V and VI; separate GLMM assessing control versus experimental status × current parasitism status: Wald, χ^2_1 = 0.86, p = 0.354). The addition or removal of workers had no influence on aggression (Wald, χ^2_2 = 0.10, p = 0.950).

Worker survival

Worker types differed in their survival (GLMM: χ^2_2 = 48.28, p < 0.0001). Remarkably, 97.2% of the 144 infected workers survived until pupal eclosion, compared with only 56.3% of the 839 uninfected workers from parasitized colonies and 69.5% of the 995 workers from unparasitized colonies. Hence, survival of tapeworm-infected workers was higher than that of their uninfected nestmates (z = 6.58, p < 0.0001, n_{colonies} = 19; **Figure 5**) or of workers from unparasitized colonies (z = 4.83, p < 0.0001, n_{colonies} = 38). Uninfected workers from parasitized colonies showed lower survival than workers from unparasitized colonies (z = −2.50, p = 0.012, n_{colonies} = 38; **Figure 5**).

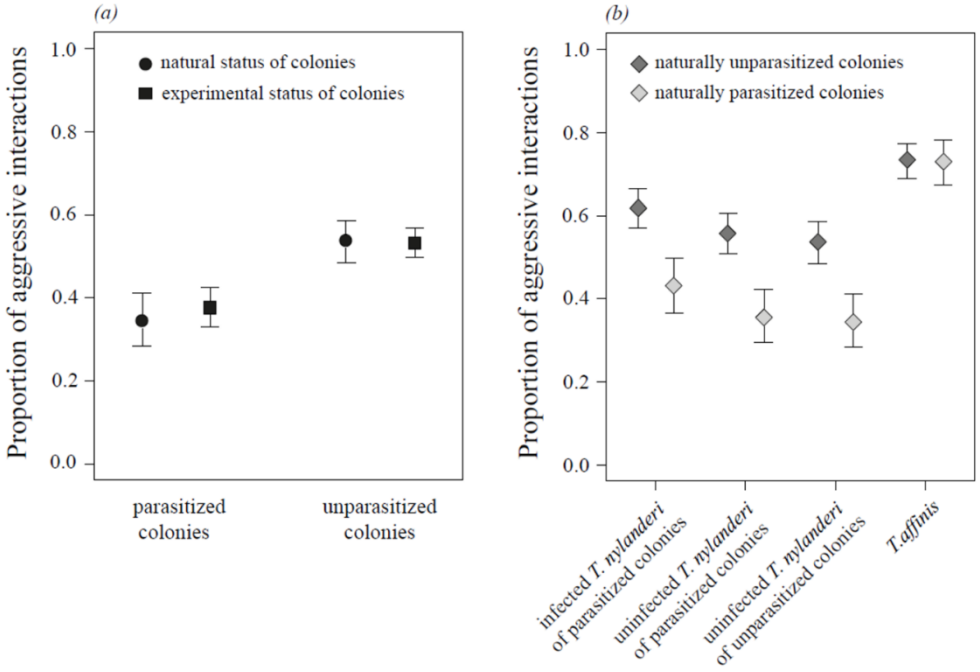

Figure 4. (***a***) Differences between parasitized and unparasitized colonies in overall aggression, in relation to their natural and experimental parasitism status. (***b***) Differences in aggression of field colonies towards four different types of opponent: infected workers of parasitized *T. nylanderi* colonies, uninfected workers of parasitized *T. nylanderi* colonies, uninfected workers of unparasitized *T. nylanderi* colonies and *T. affinis* workers. Symbols represent back-transformed GLMM estimates ± s.e).

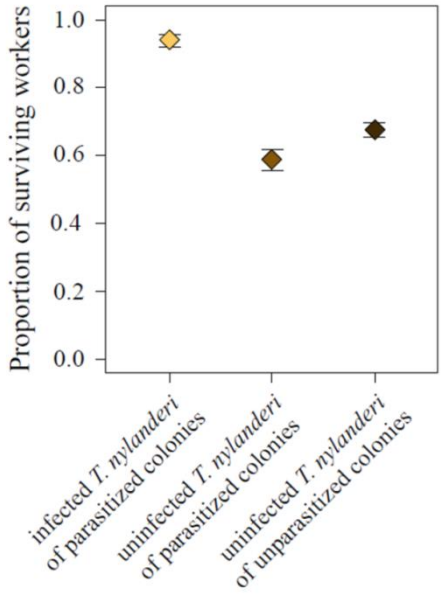

Figure 5. Survival of different worker types over the duration of 103 ± 32 days (mean ± s.d.). Symbols represent back-transformed GLMM estimates ± s.e..

Discussion

Compared with solitary species, group-living animals face a unique set of lifestyle-derived benefits and challenges. Upon infection with a parasite, infected individuals of a social group may benefit from the care provided by other group members. But social groups are also known to collectively defend themselves against exploitation by parasites (Cremer et al. 2007) and competitors (Van Zweden & D'Ettorre 2010). In this study, we investigated how the presence of infected ants affected their colony members, combining experiments on the individual (i.e. flight response, survival) and collective level (i.e. colony defence). We demonstrate that tapeworm-infected ants were less likely to flee in response to simulated nest attacks and exhibited a much higher survival while their uninfected nestmates survived less well. Moreover, the chemical profile of tapeworm-infected non-nestmates elicited more aggression in *Temnothorax* workers than uninfected non-nestmates. However, the latter responded with lower aggression towards conspecific intruders when infected ants lived in their colony. This finding indicates that the action component of nestmate recognition (i.e. behavioural response), but not the perception component (i.e. cue assessment), might be impaired.

Parasite-induced changes, whether in the individual host or the host's colony, can be due to host defences; they can either be side-effects of infection or the result of active manipulation of host phenotype by the parasite (Poulin 1995). While we did not directly assess the fitness consequences for *A. brevis* (we only simulated predator attacks), our findings suggest that the observed parasite-induced alterations in infected host individuals could benefit the parasite's survival and transmission success. First, the reduced flight response of infected *T. nylanderi* workers in response to nest attacks would presumably increase the tapeworms' transmission into the definitive avian host, which preys upon ant brood or beetle larvae. Causes of the lower escape rate might be, in addition to the lower activity of infected workers (Scharf et al. 2012b), their smaller eye and body size, and shorter legs (Trabalon et al. 2000). Second, the higher survival of infected ants would extend the parasite's time period for transmission, because predation events by woodpeckers might be rare. Interestingly, two studies support the suggestion that parasites can be responsible for the increased survival of their intermediate hosts. For instance, survival of female beetles increased upon infection with the rat tapeworm *Hymenolepis diminuta* (Hurd et al. 2001). Moreover, a recent transcriptome study on the *T. nylanderi–A. brevis* system reports a downregulation of muscle (functionality) genes and an upregulation of longevity-related genes in infected workers (Feldmeyer et al. 2016, Chapter 3), which corresponds to their inactivity and may explain increased survival.

However, the higher survival of infected *T. nylanderi* workers might not be the result of active manipulation by the parasite but rather due to the compensatory behaviour of nestmates. Indeed, a previous investigation has shown that *A. brevis*-infected workers receive ample social care, as they are more often fed and groomed

than uninfected nestmates (Scharf et al. 2012b). Similarly, *Acromyrmex* leaf-cutting ants exposed to a parasitic fungus were more often groomed by their nestmates (Walker & Hughes 2009), and hence had a better chance to survive the infection in the presence of group members (Hughes et al. 2002). Likewise, mange mite-infected wolves survived better in larger packs (Almberg et al. 2015), presumably due to the compensatory behaviours of pack-mates. While these examples do indeed represent some of the benefits of group-living, our findings also reveal costs of this lifestyle. Uninfected *T. nylanderi* ants exhibited decreased survival compared not only with infected individuals, but also with uninfected workers within unparasitized colonies. This outcome is likely to be related to the additional strain on colony resources required to care for infected ants and compensate for missing workforce because the infected individuals do not engage in colony tasks. Although the increased survival and reduced flight responses may enhance the likelihood that the parasite will complete its life cycle, it does not explain why infected workers that compromise their nestmates' survival are tolerated by host colonies. It is puzzling, as workers - both from parasitized and unparasitized colonies - were more aggressive towards infected ants, be it non-nestmates or nestmates (Scharf et al. 2012b). This suggests that workers discriminated infected from uninfected ants due to the quantitative changes in their chemical profile (Trabalon et al. 2000), although aggression did not further increase with parasite load. However, we also found that the mere presence of infected ants - either naturally occurring or experimentally introduced - triggered a reduction in aggression of parasitized colonies towards conspecifics, but not towards heterospecific intruders.

On a proximate level, aggression in ants depends on their ability to reliably recognize nestmates. Among the many models describing nestmate recognition, the adjustable threshold for recognition of conspecifics (Reeve 1989) assumes that aggression is only triggered when recognition cues perceived by the actor exceed or fall below a certain threshold (reviewed in (Fürst et al. 2011)). This threshold can be adjusted, depending on the costs or benefits of errors, which are affected by the cue diversity in the nest. As shown in *Myrmica* ants (Fürst et al. 2011), colonies harbouring more queens responded with lower aggression towards conspecifics as their cuticular hydrocarbon diversity increased due to genetic differences. In our study system, the presence of infected workers with their quantitatively different chemical profiles could similarly lead to a generally lowered aggression towards conspecifics. However, accidental or not, the induced changes could offer an explanation for the tolerance of infected workers within their colonies. For instance, integration into ant colonies is promoted by a uniform colony odour, which is achieved by an active exchange of recognition cues, either via trophallaxis or allogrooming (Boulay et al. 2000). Both behaviours are elevated in parasitized colonies (Scharf et al. 2012b) and may promote the tolerance of infected workers, related or not. On an ultimate level, reduced aggression towards conspecific competitors is expected to impair a colony's ability to defend its nest, which is a limiting resource among *Temnothorax* ants (Stroeymeyt et al. 2007). Similar costs of parasite presence were reported within the red invasive fire ants, which reduced

their foraging activity in the presence of parasitoid phorid flies, potentially resulting in a competitive disadvantage (Mehdiabadi & Gilbert 2002). In dense *T. nylanderi* populations, approximately 40% of all colonies are invaded by conspecifics searching for a new nest. This typically results in the death of the queen, but not the workers (Stroeymeyt et al. 2007). The competitive disadvantage of less aggressive, parasitized colonies could thus lead to the genetic death of the colony while the parasite's individual host survives. In conclusion, we demonstrate that the presence of tapeworm-infected individuals with drastically changed phenotypes induces behavioural alterations in uninfected group members, consequently affecting the entire social group. Additionally, our findings indicate that manipulative parasites may indirectly benefit from the social lifestyle of their hosts at the expense of other group members. This study highlights the great complexity and the impact of parasites on animal societies, and invites additional, more comprehensive exploration into the effect of parasitism in group-living hosts.

Author contributions

SB, EJ and SF designed the experiment. SB and FH collected the data. SB and EJ analysed the data. SB wrote the first draft and SB, EJ and SF revised the manuscript until completion.

Acknowledgements

[removed for privacy purposes]

Chapter 2

What are the mechanisms behind a parasite-induced decline in nestmate recognition in ants?

Sara Beros, Susanne Foitzik and Florian Menzel

Based on:
Journal of Chemical Ecology, 43, 869-880

Abstract

Social insects have developed sophisticated recognition skills to defend their nests against intruders. They do this by aggressively discriminating against non-nestmates with deviant cuticular hydrocarbon (CHC) signatures. Studying nestmate recognition can be challenging as individual insects do not only vary in their discriminatory abilities, but also in their motivation to behave aggressively. To disentangle the influence of signaling and behavioral motivation on nestmate recognition, we investigated the ant *Temnothorax nylanderi*, where the presence of tapeworm-infected nestmates leads to reduced nestmate recognition among uninfected workers. The parasite-induced decline in nestmate recognition could be caused by higher intra-colonial cue diversity as tapeworm-infected workers are known to exhibit a modified hydrocarbon signature. This in turn may broaden the neuronal template of their nestmates, leading to a higher tolerance towards alien conspecifics. To test this hypothesis, we ex-changed infected ants between colonies and analysed their impact on CHC profiles of uninfected workers. We demonstrate that despite frequent grooming, which should promote the transfer of recognition cues, CHC profiles of uninfected workers neither changed in the presence of tapeworm-infected ants, nor did it increase cue diversity among uninfected nestmates within or between colonies. However, CHC profiles were systematically affected by the removal of nestmates and addition of non-nestmates, independently from the ants' infection status. For example, when non-nestmates were present workers expressed more dimethyl alkanes and higher overall CHC quantities, possibly to achieve a better distinction from non-nestmates. Workers showed clear task-specific profiles with tapeworm-infected workers resembling more closely young nurses than older foragers. Our results show that the parasite-induced decline in nestmate recognition is not due to increased recognition cue diversity or altered CHC profiles of uninfected workers, but behavioural changes might explain tolerance towards intruders.

Keywords: cuticular hydrocarbons, nestmate recognition, parasite-induced changes, sociobiology, tapeworm, *Temnothorax nylanderi*, Formicidae

Introduction

Social insects form impressive and highly organised societies. They developed numerous lines of defence to protect their resources from exploitation and to maintain colony integrity. Nestmate recognition enables social insects to distinguish nestmates from foreign individuals and therefore represents an important, first line of defence against various intruders. Access to the nest and its resources is only given to nestmates. To this end cuticular hydrocarbons (CHCs) serve as cues for nestmate recognition (Lahav et al. 1999; Wagner et al. 2000; Dani et al. 2001; Akino et al. 2004; Dani et al. 2005). Recognition cues are heritable (Beye et al. 1998), but can be obtained from the environment as well (Heinze et al. 1996; Liang & Silverman 2000). Qualitative differences in hydrocarbons facilitate recognition between species, while conspecifics rather differ in the quantitative profile of hydrocarbons (Van Zweden & D'Ettorre 2010). The 'Gestalt' model postulates the formation of a colony-specific odor among nestmates (Crozier & Dix 1979), which may promote recognition by reducing the possibility of errors (Reeve 1989). Through the exchange of CHCs via passive physical contact, allogrooming and trophallaxis (Soroker et al. 1995; Leonhardt et al. 2016), nestmate cues are homogenised. However, a complete homogenisation may not be desired as the CHC profile contains more information beside colony identity, such as fertility (Dietemann et al. 2003), age (Wakonigg et al. 2000; Cuvillier-Hot et al. 2001), sex (Kleeberg et al. 2017) and task specialization (Greene & Gordon 2003; Kather et al. 2011). Moreover, the CHC composition shifts with parasite infections (e.g. Trabalon et al. 2000; Salvy et al. 2001; Baracchi et al. 2012; Csata et al. 2017), and nestmates use the altered signals to detect and contain infections by social immunity measures (Cremer et al. 2007; Richard et al. 2008; McDonnell et al. 2013).

Nestmate recognition is generally composed of three parts – *signalling, perception* (sensory perception and neuronal pro- cessing) and the *behavioural motivation* to act (Newey et al. 2010). However, the precise underlying mechanisms remain poorly understood and may differ between social insect species. It is commonly agreed that during perception, recognition cues of another individual are compared to one or several neuronal templates (Leonhardt et al. 2007; Newey 2011). Encountered individuals can be identified as foreign and may elicit aggression by the recipient when the other's profile differs in the amount of cues (Lenoir et al. 2001; Cini et al. 2009; Di Mauro et al. 2015) or contains novel compounds (Guerrieri et al. 2009). Aggression assays are commonly used as proxies for nestmate recognition (Roulston et al. 2003), whereby the level of aggression is assumed to reflect recognition abilities: strong behavioural responses imply good discriminatory skills, while low aggression signifies diminished nestmate recognition. However, the behavioural component of nestmate recognition is not simply a response to the level of dissimilarity in recognition cues, but rather sensitive to the context. For instance, ecological factors such as the quality of the nest and familiarity with the opponent can influence the decision to behave aggressively (Heinze et al. 1996; Tanner & Adler 2009). Moreover, the presence of nestmates (Tanner & Adler 2009) and the

number of queens in a colony as well as the relatedness between individuals (Morel et al. 1990) can be linked to different levels of aggression. Finally, individuals of the same colony can vary in their aggressive response (Newey et al. 2010). Differences in morphology and behaviour (Sturgis & Gordon 2012; Larsen et al. 2014), and consequently differential experience of workers with intruders but also nestmates can contribute to intra-colonial variation in aggression (Esponda and Gordon 2015). These examples reflect the complexity of nestmate recognition and the challenge to understand which component is altered when recognition tests show changed behavioural responses towards non-nestmates.

Here, we studied a model that allows us to disentangle the different causes of altered nestmate recognition. The ant *Temnothorax nylanderi* commonly serves as an intermediate host for the tapeworm parasite *Anomotaenia brevis* (Trabalon et al. 2000). Infected adult workers exhibit a fundamentally different phenotype including alterations in behaviour (Scharf et al. 2012b), morphology (Trabalon et al. 2000; Scharf et al. 2012b), survival (Beros et al. 2015, Chapter 1) and their CHC profile (Trabalon et al. 2000). When colonies contain tapeworm-infected nestmates, uninfected workers show reduced aggression towards non-nestmate conspecifics (Beros et al. 2015, Chapter 1). This effect can be experimentally induced by the addition and removal of infected ants (Beros et al. 2015, Chapter 1). A possible explanation for this reduced nestmate recognition is that the deviant hydrocarbon profiles of infected ants increase the quantitative diversity in recognition cues in parasitized ant colonies (i.e. having tapeworm-infected nestmates) and consequently widens the ants' neuronal template (Errard et al. 2006; Leonhardt et al. 2007). This could result in a higher tolerance towards individuals with aberrant hydrocarbon signatures. In this case, changes in aggression towards non-nestmate conspecifics should be due to a higher variation in recognition cues. This scenario resembles the higher acceptance rate of non-nestmates due to habituation to aberrant hydrocarbon signatures as shown in artificial mixed-species colonies (Errard 1994). Alternatively, aggression towards non-nestmates could change due to physiological stress caused by the parasite infection, thus reducing the behavioural motivation to act aggressively.

To differentiate whether changes in nestmate recognition are due to higher cue diversity, we experimentally studied whether potential differences in the cue signature of parasitized and unparasitized colonies are directly related to the presence of infected workers. We exchanged infected workers between colonies, and analysed hydrocarbon profiles after two months of exchange. As a control, we exchanged uninfected workers between colonies to assess effects to removal of nestmates vs. addition of non-nestmates. We expected that uninfected workers from naturally and experimentally parasitized colonies show CHC profiles different from colonies lacking infected workers. Furthermore, we were interested how worker transfer between colonies changes CHC profiles and CHC diversity within each colony. Here, we expected higher intra-colonial hydrocarbon diversity in parasitized ant colonies.

Additionally, we investigated the hydrocarbon profiles of behavioural castes (i.e. nurses, foragers) from naturally parasitized and unparasitized colonies, and our experimental colonies. The presence of infected workers in the colony might affect workers of specific behavioural castes to varying degrees. Ant scouts, foragers and nurses possess different hydrocarbon profiles (Bonavita-Cougourdan et al. 1993; Greene & Gordon 2003). Since workers of the same or of different castes regularly exchange CHCs during trophallaxis and allogrooming (Vienne et al. 1995; Leonhardt et al. 2016), worker castes which differ most strongly from infected workers might show the strongest CHC changes after exposure to infected workers. On the other hand, infected workers are long-lived (Beros et al. 2015, Chapter 1) and foragers are usually older than nurses (Mersch et al. 2013). Hence, we either expected to find more age-related similarities between foragers and infected workers, or alternatively, nurses could resemble infected workers due to their frequent social interactions and spatial proximity (Scharf et al. 2012b).

Methods

Study system, colony collection and maintenance

Temnothorax nylanderi occurs in deciduous forests throughout Europe and inhabits small, protected cavities in acorns and sticks on the forest floor. Ant colonies were collected from different locals in the vicinity of the cities Mainz and Rüdesheim (Germany) from May to June 2014 (**Table S1**). Colonies were transported in ziploc bags to the laboratory and transferred to artificial observation nests (i.e. pre-cut cavity between two glass slides; 50 × 10 × 3 mm^3), which were housed in plastered boxes (100 × 100 × 30 mm^3). Ants were kept in a climate chamber at 20°C : 16°C (12 L : 12D cycle) and were provided with honey and pieces of crickets once a week. Access to water was unlimited and nest boxes were moistened if necessary. Colonies were counted after moving to the new nest. We included the number of queen, brood and workers - differentiating between brown and yellow workers in parasitized colonies (Beros et al. 2015, Chapter 1).

Dataset a – field data: cuticular hydrocarbon profiles of field colonies

Tapeworm-infected *T. nylanderi* workers possess a modified CHC profile (Trabalon et al. 2000), and frequent allogrooming and trophallaxis could alter the CHC profile of their nestmates (Scharf et al. 2012b), potentially explaining the hampered nestmate recognition of parasitized colonies (Beros et al. 2015, Chapter 1). To analyse this, we sampled an uninfected forager, an uninfected nurse each from 21 naturally parasitized and 23 naturally unparasitized colonies, and sampled one infected worker from 21 parasitized colonies. Uninfected workers were selected based on their spatial position (Modlmeier et al. 2012): foragers were collected from outside the nest, whereas nurses were defined as those that cared for the brood inside the nest. *Anomotaenia brevis*-infected workers can be reliably recognized by the bright yellow coloration of their cuticle (Beros et al. 2015, Chapter 1). However, it is

difficult to assign them to either the forager or the nurse caste, because infected workers engage less in colony tasks (Scharf et al. 2012b). When assigned according to their position on the brood pile (Scharf et al. 2012b), infected workers take after nurses, but when grouped by age they resemble more the older foragers.

Dataset b – experimental data: manipulation of ant colony parasitism status

Given the low aggression towards non-nestmate conspecifics (Foitzik et al. 2007; Beros et al. 2015, Chapter 1), *Temnothorax nylanderi* colonies can be easily manipulated by adding or removing workers (Beros et al. 2015, Chapter 1). Indeed, within areas of high population density, unrelated colonies will regularly merge (Foitzik & Heinze 1998; 2001). To investigate the influence of infected workers on the hydrocarbon profile of their nestmates, we manipulated colony composition. For this worker exchange experiment, we used 80 queenright colonies from a single population (see **Table S1**). Twenty parasitized and 60 unparasitized colonies were assigned to four different treatments (I – IV); (**Figure 6**), in which we either removed or added workers. We removed all yellow workers (3–14 individuals) from 20 parasitized colonies (treatment I: originally parasitized, currently unparasitized; donor colonies). Workers originating from a single donor colony were then added to an unparasitized receiver colony (treatment III: originally unparasitized, currently parasitized; n = 20). Colonies of treatment II and IV served as controls for the removal and addition of ant workers. Hence, we removed workers from a total of 20 unparasitized colonies (3–13 workers; treatment II: originally unparasitized, currently unparasitized; donor colonies) and added them to an alien unparasitized colony (treatment IV: originally unparasitized, currently unparasitized; receiver colonies, n = 20). We chose to remove and add only nurses as they are more likely to remain inside the nest. This allowed us the comparison between colonies of treatment III (infected workers added) and IV (uninfected workers added). Colonies that received non-nestmates (treatment III & IV) were matched in terms of colony size and the added number of workers. Moreover, we standardized the intra-colonial infection rate to ~13% infected workers per colony (12.72 ± 0.01%, mean ± s.d.), which resembles the infection rate found in the field (Scharf et al. 2012b).

To facilitate manipulations, colonies were anaesthetised with CO_2 for a few seconds before workers were either removed or added. Before introducing workers to a new colony, all ants, including infected workers (n = 291) were tagged with coloured wires (ELEKTRISOLA, 0.025 mm) to be reliably identified and distinguished from native colony members. After manipulation, experimental colonies of all four treatments were kept at 8 °C for ten days to reduce the likelihood of worker rejection. During this time, receiver colonies (treatment III & IV) were checked on a daily basis, and if necessary, expelled wire-marked ants were reintroduced by carefully pushing them back to the colony with forceps and closing the nest entrance

with a tissue until the next day. After ten days, colonies were moved to a climate chamber at 20 °C for the next 46 days such that the experimental manipulation lasted for a total of 56 days.

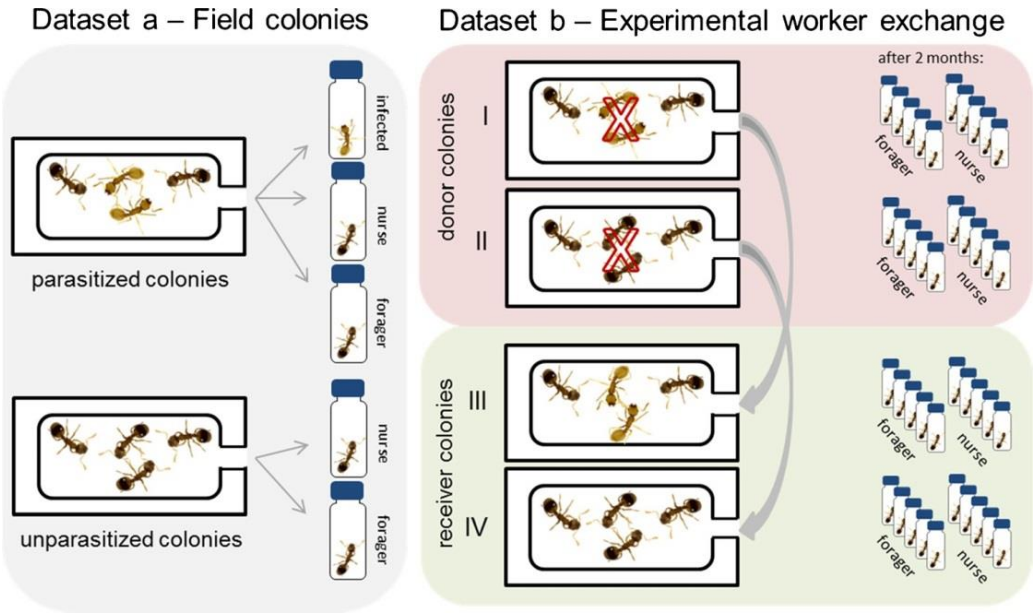

Figure 6. Data sampling and experimental set-up. From field colonies we sampled one nurse and one forager from each colony type (unparasitized & parasitized) and additionally an infected worker from parasitized *T. nylanderi* colonies. All infected workers from naturally parasitized colonies (i.e. treatment I) were removed and added to naturally unparasitized colonies of treatment III. As controls we used naturally unparasitized colonies assigned to treatment II from which we removed identical numbers of workers and added them to naturally unparasitized colonies of treatment IV. Two months after the exchange, we sampled from each colony five native foragers and five native nurses.

Dataset b – experimental data: ant sampling for chemical analyses

The experiment ended 56 days after manipulation when we sampled 10 native, uninfected workers (five nurses and five foragers) from each colony. Ants were individually frozen in glass vials at −20°C. For our chemical analyses of individuals from treatment III (n = 16) and IV (n = 16), we only used those colonies in which non-nestmates were well integrated into the colony. From six donor colonies ($n_{\text{treatment I}}$ = 5; $n_{\text{treatment II}}$ = 1), we did not take samples for chemical analyses as these colonies consisted of less than ten workers, which showed no behavioural differentiation and hence impeded us to reliably distinguish workers according to their behavioural task.

Chemical analyses via GC-MS

CHC of single ants were extracted in ~0.5ml of hexane for 10 min. During extraction, 100ng *n*-C18 was added as internal standard (Mas et al. 2009). The extracts were concentrated to ~20µl, and we injected 5µl into a gas chromatograph coupled to a mass selective detector (GC-MS) (Agilent Technologies, GC: Agilent 7890A, MSD: Agilent 5975) equipped with a HP5-MS column (30m × 0.25mm; coating: 0.25µm). Injection was performed in the split-less mode at 250°C, using helium as carrier gas with a constant flow of 1.2ml min. Oven temperature was set at 150°C for 3 min, followed by a two-step temperature increase from 150 to 250°C at 30°C min and 250–300°C at 2°C min, where the temperature remained constant for 2 min. Masses were scanned in the range 40–500amu at an ionization voltage of 70eV. Data were acquired using the software MSD Chemstation E.02.02 (Agilent). We quantified the relative amounts of CHC based on peak areas, and made sure that none of the sample runs contained any overcharged peaks. Hydrocarbons were identified using their retention indices and diagnostic ions. Using this method, a total of 108 samples from field colonies and 526 samples from the worker ex- change experiment were analysed.

Statistical analyses

For both datasets we assessed differences in CHC profiles between (*a*) infected and uninfected workers from field colonies, and (*b*) between foragers and nurses from the four experimental colonies, using a permutational multivariate analysis of variance (PERMANOVA, 999 permutations) based on the Bray-Curtis similarity in the software Primer 6.0 & PERMANOVA (Primer-E Ltd.). The model on the field data included the worker type (infected worker, uninfected nurse, uninfected forager) and the field parasitism status (parasitized, unparasitized), with interactions allowed. For dataset b, we constructed a model including behavioural caste (nurse, forager), experimental treatment (donor, receiver colonies) and exchanged worker type (infected, uninfected worker) as well as their interactions as fixed predictors. To account for colony identity, we added colony ID as a random factor, which was nested in experimental treatment and exchanged worker type.

In both datasets we additionally performed analyses on the abundance of specific substance classes and hydrocarbon characteristics. To elucidate differences in CHC composition of infected and uninfected workers from the field data (dataset *a*), and to evaluate whether the addition and removal of infected workers affected the CHC composition of nestmates (dataset *b*), we analysed in each dataset the following traits, each as the dependent variable in separate linear mixed models (LME): (*i*) the mean chain-length of CHCs, (*ii*) the total amount of CHCs, (*iii*) the proportion of straight-chain alkanes (*n*-alkanes) as well as the proportion of (*iv*) mono-, (*v*) di- and trimethyl alkanes; the two hydrocarbon classes were pooled since *T. nylanderi* only possesses five trimethyl alkanes. For the field data, we used worker type (infected, nurse, forager) and field parasitism status (parasitized, unparasitized) as fixed predictors, with interactions allowed.

For the worker exchange experiment, we included behavioural caste (nurse, forager), the experimental treatment (donor, receiver colony) and exchanged worker type (infected, uninfected workers) as fixed predictors, with interactions allowed. Again, the colony ID (nested in experimental treatment and exchanged worker type) was entered as a random factor.

Finally, we analysed within-colony variation for field colonies (dataset *a*), and between- and within-colony variation among experimental colonies (dataset *b*). For each field colony, we calculated the Bray-Curtis distance between forager and nurse of unparasitized colonies and compared it to the average distance between forager and nurse, forager and infected worker, and nurse and infected worker of parasitized colonies using a *t* test. For experimental colonies, between-colony variation was compared between the four treatments using PERMDISP (Primer). To analyse within-colony variation, we calculated the average distance from the colony centroid (output of the PERMDISP command in Primer). We included both foragers and nurses, but repeated the analysis for nurses and foragers separately to control for effects of caste differences. These values (one per colony, n = 61) were then compared between treatments using a linear model. All linear models were constructed in R v 2.15.2 (R Core Team 2012).

Results

We found a total of 37 saturated CHC peaks on the cuticle of *T. nylanderi* ants (**Table 1**), consisting of seven *n*-alkanes, 30 monomethyl, 20 dimethyl as well as five trimethyl alkanes. All hydrocarbons were shared among the three worker types (infected workers, nurses, foragers), with differences in the relative abundance of 28 hydrocarbons (**Table 1**). Foragers differed strongly from infected workers and nurses (**Table 1**), whereas the latter two were more similar. Yet, we found significant differences between infected workers and nurses in one n-alkane and seven methyl-branched alkanes (**Table 1**).

Chemical analyses of field colonies

As infected workers are frequently groomed by their uninfected nestmates and grooming leads to CHC transfer (Soroker et al. 1995), we firstly investigated whether the hydrocarbon profile of uninfected workers was affected by the parasitism status of the colony. Interestingly, the chemical profile of uninfected foragers and nurses from parasitized colonies did not differ from workers of the same caste from unparasitized colonies (**Table S2**). However, worker type had a strong influence on the CHC profile, showing differences between infected workers, nurses and foragers (PERMANOVA: Pseudo-F_2 = 8.96, $p < 0.001$). The CHC profile of foragers differed most strongly from infected workers (PERMANOVA pairwise contrast: t = 4.01, p = 0.001) and nurses (t = 2.99, p = 0.001), but also between infected ants and nurses (t = 1.79, p = 0.015; **Figure 7**).

Table 1. Cuticular hydrocarbons of *Temnothorax nylanderi* workers from field colonies (parasitized and unparasitized colonies).

Peaks	*Substance*	*Kovats index*	*Relative abundance, mean ± SE (%)*			*p value*	
			Infected (I)	**Nurses (N)**	**Foragers (F)**	**I – N - F**	**I - N**
1	nC25	2500	0.38 ± 0.05	0.52 ± 0.08	0.59 ± 0.08		
2	nC26	2600	0.12 ± 0.01	0.14 ± 0.02	0.14 ± 0.01		
3	nC27	2700	5.95 ± 0.69	5.46 ± 0.73	4.06 ± 0.27		
4	*11;13-MeC27*	2731	0.63 ± 0.09	0.63 ± 0.07	1 ± 0.11	*0.046*	
5	*7-MeC27*	2741	0.08 ± 0.01	0.07 ± 0.01	0.1 ± 0.01	*0.008*	
6	**5-MeC27**	2749	0.41 ± 0.05	0.32 ± 0.04	0.42 ± 0.06		**0.028**
7	11,15-;11,17-DiMeC27	2762	0.1 ± 0.02	0.08 ± 0.01	0.09 ± 0.01		
8	**3-MeC27**	2772	3.24 ± 0.45	2.53 ± 0.27	2.86 ± 0.23		**0.006**
9	nC28	2800	0.45 ± 0.05	0.3 ± 0.02	0.38 ± 0.03		
10	*6-MeC28*	2842	0.05 ± 0.01	0.04 ± 0.01	0.06 ± 0.01	*0.009*	
11	*4-MeC28*	2857	0.43 ± 0.06	0.32 ± 0.03	0.46 ± 0.04	*0.042*	
12	***nC29***	2900	1.52 ± 0.18	0.91 ± 0.08	1.04 ± 0.07	*0.014*	**0.002**
13	*11;13;15-MeC29*	2932	2.09 ± 0.24	2.24 ± 0.24	3.93 ± 0.45	*0.028*	
14	*7-MeC29*	2941	0.23 ± 0.03	0.19 ± 0.02	0.24 ± 0.02	*>0.0001*	
15	***5-MeC29***	2951	0.28 ± 0.04	0.18 ± 0.03	0.21 ± 0.02	*0.029*	**0.006**
16	*11,17-DiMeC29*	2961	0.49 ± 0.09	0.36 ± 0.04	0.54 ± 0.06	*0.011*	
17	**3-MeC29**	2975	0.73 ± 0.11	0.49 ± 0.09	0.55 ± 0.05		**0.002**
18	***5,15-DiMeC29***	2980	0.2 ± 0.05	0.11 ± 0.01	0.2 ± 0.05	*0.003*	**0.014**
19	***3,15-DiMeC29***	3012	0.4 ± 0.05	0.27 ± 0.03	0.39 ± 0.05	*0.009*	**0.007**
20	*11;12;13;14;15-MeC30*	3031	0.26 ± 0.03	0.29 ± 0.03	0.52 ± 0.06	*0.021*	
21	*3,7,11;3,7,15-TriMeC29*	3037	0.12 ± 0.03	0.08 ± 0.01	0.13 ± 0.03	*<0.0001*	
22	4;2-MeC30; 11.15;13.17-DiMeC30	3061	0.12 ± 0.02	0.13 ± 0.02	0.25 ± 0.04		
23	*3-MeC30*	3072	0.07 ± 0.02	0.06 ± 0.03	0.06 ± 0.01	*0.021*	
24	*nC31*	3100	0.13 ± 0.03	0.06 ± 0.01	0.09 ± 0.01	*0.020*	
25	*11;13;15-MeC31*	3131	1.25 ± 0.18	1.44 ± 0.18	2.89 ± 0.35	*<0.0001*	
26	*11,15-;13,17-DiMeC31; 9,17-DiMeC31; 11,15,19-TriMeC31*	3152 3162 3180	1.15 ± 0.25	1.02 ± 0.12	1.81 ± 0.19	*>0.0001*	
27	*3,15; 3,17-DiMeC31*	3202	0.22 ± 0.04	0.18 ± 0.02	0.28 ± 0.04	*0.0417*	
28	*12;14;16-MeC32*	3231	0.08 ± 0.02	0.1 ± 0.02	0.23 ± 0.03	*<0.0001*	
29	***8,16-DiMeC32***	3237	0.52 ± 0.1	0.35 ± 0.05	0.66 ± 0.1	*0.043*	**0.049**
30	*12,16-DiMeC32*	3255	0.14 ± 0.05	0.11 ± 0.01	0.26 ± 0.03	*<0.0001*	
31	*13;15;17-MeC33*	3330	0.4 ± 0.09	0.48 ± 0.07	1.21 ± 0.16	*<0.0001*	
32	*13,17-;13,19-DiMeC33*	3355	0.93 ± 0.24	0.89 ± 0.09	1.69 ± 0.18	*0.0001*	
33	*9,17-;9,19-DiMeC33*	3361	0.46 ± 0.1	0.56 ± 0.09	1.11 ± 0.16	*>0.0001*	
34	*11,15,19-TriMeC33*	3379	0.32 ± 0.12	0.26 ± 0.03	0.44 ± 0.05	*0.005*	
35	*nC34*	3400	0.09 ± 0.02	0.08 ± 0.01	0.13 ± 0.02	*0.033*	
36	*11/13/15,17/19/21-DiMeC35**	3558	1.65 ± 0.4	1.57 ± 0.15	3.1 ± 0.37	*>0.0001*	
37	*11,15,19-TriMeC35*	3575	0.25 ± 0.07	0.21 ± 0.03	0.36 ± 0.05		

*combination of 1st and 2nd methyl group unknown

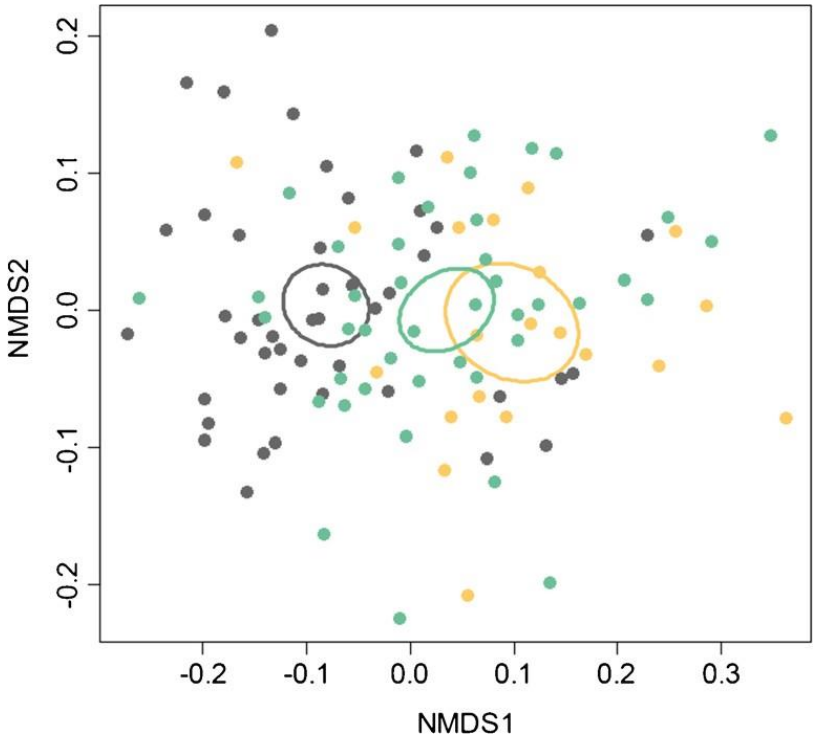

Figure 7. NMDS plot showing cuticular hydrocarbon resemblance of uninfected foragers (grey dots), uninfected nurses (green dots) and tapeworm-infected *T. nylanderi* (yellow dots). Resemblance is based on the Bray-Curtis Similarity and stress value is 0.14. Ellipses represent 95% of confidence interval.

Compared to foragers, the chemical profile of infected workers (LME: $t = -4.73$, $p < 0.001$) and nurses (LME: $t = -4.24$, $p < 0.001$) was composed of shorter-chained hydrocarbons (**Figure 8a**). Moreover, infected workers and nurses had both proportionally more *n*-alkanes (LME: infected: $t = 4.37$, $p < 0.001$; nurses: $t = 4.27$, $p < 0.001$), but less mono- and di/trimethyl alkanes than foragers (LME: monomethyl alkanes $_{\text{infected vs. forager}}$: $t = -2.18$, $p = 0.032$; monomethyl alkanes $_{\text{nurses vs. forager}}$: $t = -2.00$, $p = 0.048$; di/ trimethyl alkanes $_{\text{infected vs. forager}}$: $t = -3.21$, $p = 0.002$; di/ trimethyl alkanes $_{\text{nurses vs. forager}}$: $t = -3.25$, $p = 0.002$); (**Figure 8c**). On the other hand, foragers had generally more hydrocarbons on their cuticle than nurses ($t = 3.17$, $p = 0.002$), while infected workers did neither differ from foragers ($t = 1.32$, $p = 0.189$) or nurses ($t = -1.23$, $p = 0.220$; **Figure 8b**). Despite these distinct differences between worker types, parasitized and unparasitized colonies did not differ in the average chemical distance between workers (*t* test: $t_{30.9} = 1.31$, $p = 0.20$).

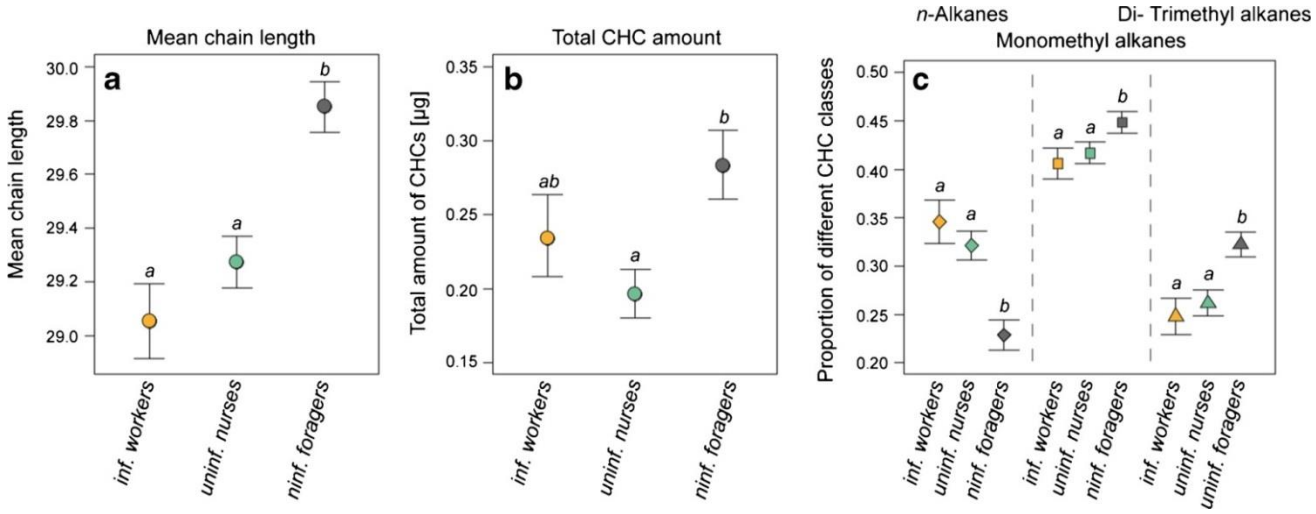

Figure 8. Cuticular hydrocarbon (CHC) profiles of different *Temnothorax nylanderi* ant worker types from field colonies (parasitized and unparasitized colonies). (**a**) mean chain length of hydrocarbons, (**b**) total amounts, (**c**) proportion of *n*-alkanes (diamonds), monomethyl alkanes(squares) as well as di/trimethyl alkanes (triangles). Colours show the three worker types (infected workers = yellow, uninfected nurse = green, uninfected foragers = grey). Plots with same letters are not significantly different.

Chemical analyses of worker exchange colonies

Contrary to our expectations, only few differences in hydrocarbon chemistry were observed between colonies that received infected or uninfected ants (**Table S3**). Thus, the infection status of added or removed workers played a minor role (all $p >$ 0.29; **Table S3**). In contrast, most differences were found between the two behavioural castes (i.e. nurses and foragers) and the experimental treatment (i.e. donor and receiver colonies), and/or the interaction of those two factors (**Table S3**). After the experimental exchange of *T. nylanderi* workers, foragers and nurses still showed clear differences in their chemical profiles (PERMANOVA: Pseudo-F_1 = 20.98, $p < 0.001$), and this difference partially depended on the experimental treatment (interaction: behavioural caste x experimental treatment: $F_1 = 2.98$, $p = 0.021$). Foragers and nurses from receiver colonies differed more in their hydrocarbon profiles ($t = 3.774$, $p = 0.001$) than those from donor colonies ($t = 3.194$, $p = 0.001$); (**Figure S1**). Hydrocarbon traits differed mainly between donor and re- ceiver colonies. Ant workers from receiver colonies had higher absolute amounts of hydrocarbons ($t = 2.02$, $p = 0.048$; **Figure 9b**), and foragers had marginally higher absolute amounts than nurses (LME: $\chi^2_1 = 3.76$, $p = 0.053$). The proportion of *n*-alkanes was generally lower in workers from receiver colonies ($t = -2.65$, $p = 0.010$), but differences in the proportion of mono- and di/trimethyl-alkanes between the experimental colonies depended also on the behavioural caste

(monomethyl: behavioural caste x experimental treatment: $\chi^2_1 = 7.80$, $p = 0.005$, di−/trimethyl: monomethyl: behavioural caste x experimental treatment: $\chi^2_1 = 5.56$, $p = 0.018$). Hence, nurses from receiver colonies had the lowest amount of monomethyl alkanes ($t = -6.83$, $p < 0.001$; **Figure 9c**), but the highest of di/trimethyl alkanes ($t = 3.44$, $p < 0.001$; **Figure 9c**). The mean hydrocarbon chain length differed between behavioural castes (LME: $\chi^2_1 = 5.15$, $p = 0.023$) and experimental treatments (LME: $\chi^2_1 = 26.83$, $p < 0.001$; **Figure 9a**). Similar to the effects in the field colony analyses, nurses had shorter- chained hydrocarbons ($t = -2.27$, $p = 0.024$), and colonies that received foreign workers had generally longer-chained hydrocarbons ($t = 5.08$, $p < 0.001$).

The infection status of added or removed workers only weakly affected the abundance of di−/trimethyl alkanes (LME: $\chi^2_1 = 5.25$, $p = 0.022$) or mean CHC chain length (LME: $\chi^2_1 = 4.19$, $p = 0.041$), and did not influence any other parameters. Uninfected workers from colonies that once contained infected nestmates or received infected non-nestmates had overall shorter-chained hydrocarbons ($t = -2.03$, $p = 0.047$) and shorter-chained di−/trimethyl alkanes ($t = -2.26$, $p = 0.028$) and overall shorter-chained hydrocarbons ($t = -2.26$, $p = 0.028$; **Figure 10**). Between-colony chemical variation (based on five foragers and five nurses per colony) was highest in colonies from which infected workers had been removed ('donor colonies'; treatment I). They were more variable than those from which uninfected workers had been removed ('donor colonies'; treatment II) (PERMDISP: overall $F_{3,523} = 15.75$, $p = 0.001$; I vs II: $t = 3.35$, $p = 0.001$). The variation was the lowest in the two receiver treatments (III and IV: $t = 3.1$, $p < 0.002$ for each pairwise comparison to donor treatments), which did not differ from each other (III vs. IV: $t = 0.15$, $p = 0.88$). Analyses on between-colony variation based on only foragers or only nurses yielded similar results. In contrast, within-colony variation (based on foragers and nurses) did not differ between treatments (LM: $F_3 = 0.42$, $p = 0.74$), and the same was true for variation concerning only foragers or only nurses.

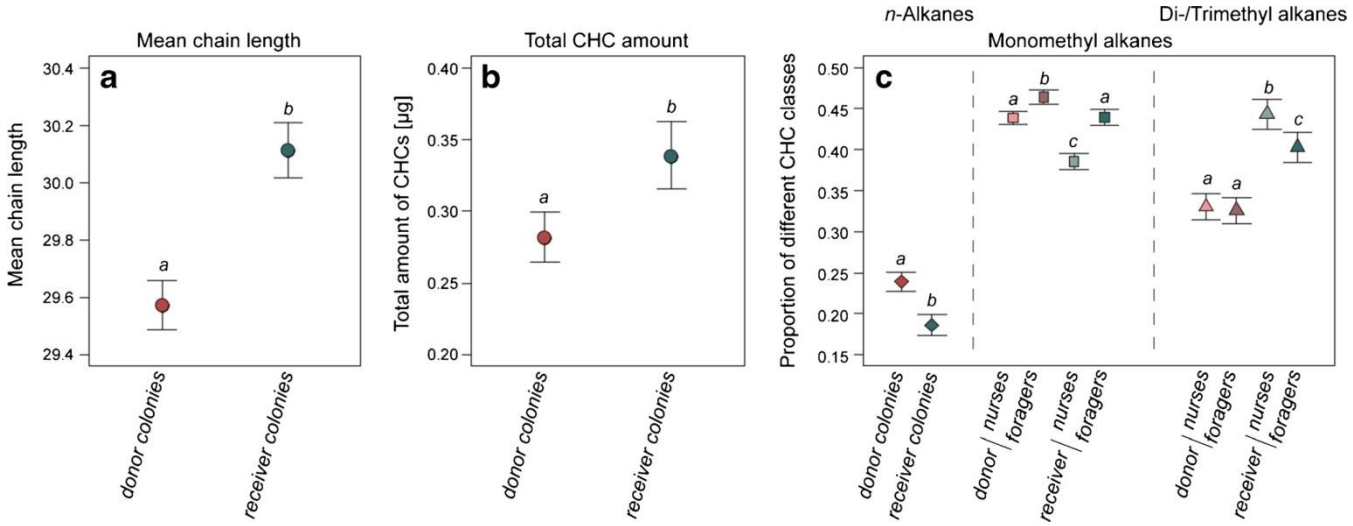

Figure 9. Cuticular hydrocarbon (CHC) profiles of *Temnothorax nylanderi* ant colonies and individuals subjected to different *experimental treatments. (***a***)* mean chain length of hydrocarbons, (**b**) total amounts, (**c**) proportion of *n*-alkanes (diamonds), monomethyl alkanes (squares) as well as di/trimethyl alkanes (triangles). Colours show the two experimental treatments (donor colonies = red, receiver colonies = green). Nurses (light red & green) differed from foragers (dark red & green) only for the complex CHCs. Plots with different letters differ significantly from each other.

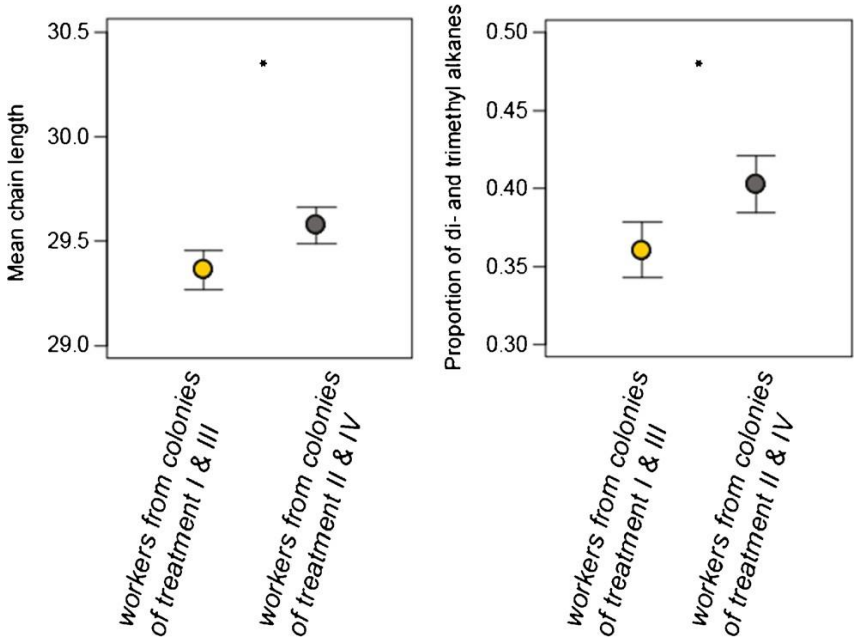

Figure 10. Cuticular hydrocarbon (CHC) profiles of *Temnothorax nylanderi*, showing mean chain length of hydrocarbons and the proportion of di-and trimethyl alkanes of uninfected workers in relation to the addition of infected (yellow coloured) and uninfected conspecific workers (grey coloured).

Discussion

In this study we investigated experimentally whether odour mixing between infected and uninfected ant workers leads to a more diverse colony odour in parasitized colonies. This increase in intra-colonial hydrocarbon variance could reduce inter-colonial differences and therefore explain the reduced aggression exhibited by parasitized colonies (Beros et al. 2015, Chapter 1). Contrary to our predictions, parasitized colonies were not chemically more diverse. Despite frequent grooming and trophallaxis, behaviours known to mediate hydrocarbon transfer (Vienne et al. 1995), the presence of infected workers did not affect the hydrocarbon profiles of uninfected workers. Interestingly, the removal of nestmates and addition of non-nestmates rather than the infection status of the exchanged workers affected the colony odour. This stands in stark contrast to our earlier behavioural experiments (Beros et al. 2015, Chapter 1), in which the removal and addition per se did not change colony aggression, whereas the infection status of the removed or added workers did. One possible explanation for the radical change in chemical signature in response to worker exchange is that ants increase the production of colony-specific recognition cues, which changes the overall colony composition. Yet, overall our findings suggest that aggression in our study species is not simply associated with differences in CHC profiles. A similar conclusion has been reached for the harvester ant *Pogonomyrmex barbatus*, where chemical and behavioural differences between task groups are stronger than those between colonies (Sturgis & Gordon 2012).

No effect of infected workers on CHC profiles and cue diversity

Uninfected workers from parasitized colonies are less aggressive than those from unparasitized colonies (Beros et al. 2015, Chapter 1). Ants from artificially mixed and hence, chemically diverse colonies show particularly low aggression towards non-nestmates (Errard et al. 2006). As such, we hypothesised that the reduced aggression of workers from parasitized colonies of *T. nylanderi* is due to the aberrant profile of infected workers, which should lead to higher cue diversity within these colonies.

The '*Gestalt*' model suggests that frequent interactions (e.g. allogrooming, trophallaxis) between nestmates allow a continuous transfer of hydrocarbons and therefore lead to the formation of a colony-specific odour. In artificial mixed colonies (Stuart 1988; Errard 1994), workers of both species gradually acquired some hydrocarbons from their heterospecific nestmates, probably leading to a broader colony signature and in turn increasing tolerance towards alien individuals. The same is true for naturally mixed colonies of slavemaking ants, in which both slavemakers and hosts obtain hydrocarbons from each other (Brandt et al. 2005; Bauer et al. 2010). As workers of parasitized *T. nylanderi* colonies show high allogrooming and trophallaxis rates towards their tapeworm-infected nestmates (Scharf et al. 2012b), which possess a distinct hydrocarbon profile (Trabalon et al. 2000), the cue signature of uninfected workers in our colonies might have shifted towards those of infected workers. However, the hydrocarbon profiles of

uninfected nestmates did not change with parasitism status of the colony, nor did workers from either colony type differ in their average chemical distance. These findings imply that the behavioural changes observed in uninfected nestmates of infected individuals (Scharf et al. 2012b; Beros et al. 2015, Chapter 1), in particular the reduction in aggression, are determined by factors other than hydrocarbon chemistry and colony odour. Chemical compounds other than CHCs can also modify the behaviour of social insects. Nest volatiles such as short-chain alkanes have been shown to decrease aggression in *Camponotus fellah* ant workers (Katzav-Gozansky et al. 2008), and also in a parasitic context they play an important role as olfactory cues. The odour of mice infected with transmissible stages of the malaria parasite, *Plasmodium chaubaudii*, enhances the attraction of mosquitos due to the production and release of volatiles (De Moraes et al. 2014). Volatiles were further involved in the interactions between microorganisms and insects via microbial volatile organic compounds (Davis et al. 2013). Thus, the release of volatiles in tapeworm-infected individuals might similarly influence colony aggression, opening up new avenues for research. Moreover, members of social insect colonies are known to alter their behaviour when confronted with infected peers and pathogens (Cremer et al. 2007), and may be even able to weigh the risk of disease transmission and damage to their colony (e.g. Heinze & Walter 2010; Rueppell et al. 2010; Bos et al. 2012). The behavioural repertoire is diverse and ranges from antagonistic responses (Richard et al. 2008; Baracchi et al. 2012) to more social care such as increased self- and allogrooming (Aubert & Richard 2008; Walker & Hughes 2009; Konrad et al. 2012a), which can be observed in tapeworm-parasitized colonies of *T. nylanderi* as well (Scharf et al. 2012b).

More investigations are required to understand whether the deviant hydrocarbon profile of infected ants (Trabalon et al. 2000) induces the behavioural changes in their nestmates and whether the altered behaviours have implications for social immunity. A recent study demonstrates that the detection of CHCs from immune-stimulated and diseased honeybees can initiate an immune response in queens (López et al. 2017). Likewise it is possible that the interactions with tapeworm-infected workers alter their nestmates' physiology. Indeed, the gene expression pattern in brains of uninfected *T. nylanderi* workers from parasitized colonies show differences to those living in unparasitized colonies (Feldmeyer et al. 2016, Chapter 3). In addition, the higher mortality rate of uninfected workers from parasitized colonies (Beros et al. 2015, Chapter 1) implies that caring for infected workers (Scharf et al. 2012b) is associated with physiological costs, which might weaken the ants' aggression level.

Effect of removal and addition of workers

Although infection status of exchanged workers strongly affected aggression (Beros et al. 2015, Chapter 1), we show that it does not influence the hydrocarbon profiles of nestmates. Rather the removal and addition of workers per se had a strong and complex impact on the CHC profile. Colonies that received non-nestmates showed

more di−/trimethyl alkanes, but less *n*-alkanes and monomethyl alkanes in their profiles, higher average chain lengths, and higher absolute CHC quantities. As we only analysed the profiles of native workers, the chemical changes we observed suggest that these workers responded to the presence of foreign individuals by producing more hydrocarbons, and in particular more di−/trimethyl alkanes. These more complex hydrocarbons have been shown to be utilized by bees and ants for nestmate recognition (Dani et al. 2001; Akino et al. 2004; Dani et al. 2005; Guerrieri et al. 2009; Sturgis & Gordon 2012). Further trimethyl alkanes are more easily learned by ants compared to *n*-alkanes and monomethyl alkanes (Van Wilgenburg et al. 2011). It is therefore possible that the enhanced production of these structurally more complex hydrocarbons is an adaptive response to signal colony identity more clearly (Sturgis & Gordon 2012), thereby ensuring that altruistic behaviours are directed only to related individuals. The longer chain length may be explained by the need to maintain the viscosity of the CHC layer despite a lower percentage of *n*-alkanes (Gibbs 1995; Gibbs & Pomonis 1995). Surprisingly, the changes in the proportion of hydrocarbon classes due to worker exchange were not accompanied by changes in hydrocarbon variation within colonies. However, between-colony variation was affected. Supposedly, the addition of 13% non-nestmates blurred chemical differences between colonies such that inter-colony variation was lower in receiver colonies.

Differentiation between infected workers, nurses and foragers

CHCs of *T. nylanderi* workers were comprised of a mixture of linear and methyl-branched alkanes, which differed in their relative abundance among nurses, foragers and infected workers. These analyses support earlier findings on CHC changes with parasite infections (e.g. Trabalon et al. 2000; Salvy et al. 2001; Richard et al. 2008; Csata et al. 2017), which also revealed shifts in the relative abundance rather than the absence or presence of certain hydrocarbons. Yet, in contrast to Trabalon and colleagues (2000), we differentiated between worker castes and thus can demonstrate that the CHC profiles of infected workers differ more strongly from foragers than from nurses. Moreover, the difference between nurses and foragers was greater than that between nurses and infected workers. As the presence of infected workers did not affect the hydrocarbon profiles of other workers, the chemical resemblance of nurses and infected workers is likely not due to the exchange of hydrocarbons. It is more likely that similarities in physiology, behaviour or spatial location in the nest might lead to profile convergence. Due to their high survival rates infected workers likely represent the oldest worker caste in the nest (Beros et al. 2015, Chapter 1).

Nurses are usually the youngest workers in a colony, while the older workers become foragers (Mersch et al. 2013). Thus, chemical differences between foragers and nurses are not solely due to age – otherwise, infected workers should have resembled foragers more than nurses. The deviant profile of foragers may rather reflect acclimation to their external environment. Being frequently outside the nest foragers

experience more desiccation stress. In several social insect species foragers carry more *n*-alkanes than nurses (Wagner et al. 1998; Martin & Drijfhout 2009; Kather et al. 2011; Sturgis & Gordon 2012; Pamminger et al. 2014), which protect better against desiccation than methyl-branched alkanes (Gibbs & Pomonis 1995). In this study, however, *Temnothorax* foragers had relatively less *n*-alkanes, but carried generally more hydrocarbons and also more information-rich methyl-branched hydrocarbons (Akino et al. 2004). One simple reason could be the stable environmental conditions in the laboratory. A biological more relevant argument is that foragers may need more recognition cues since they are more likely to encounter non-nestmates than nurses. In addition, nurses and infected workers may have acquired hydrocarbons from the brood, which mainly contain *n*-C27 (60–70% of the profile) and 3-MeC27 (12%; unpublished data). These two substances are common in all three behavioural castes, but particularly abundant in infected workers. The profiles of infected workers differed in the relative abundance of eight compounds from uninfected workers. Interestingly, four out of these eight hydrocarbons (i.e. 5-MeC27, 3-MeC27, 5-MeC29 and 3-MeC29) were found to explain inter-colonial aggression and thus are thought to be important in nestmate recognition in a North American *Temnothorax* species (Jongepier & Foitzik 2016). We found that, overall, worker aggression in parasitized *T. nylanderi* is, however, reduced in the presence of infected workers, but the difference in CHC profile still may explain the occasional attacks against infected workers within their colonies (Scharf et al. 2012b). Our study provides experimental evidence that the presence of infected workers does not lead to higher intra-colonial variation in CHC profiles. Thus, the previously observed decrease in aggression due to the presence of infected workers in the nest must be caused by other factors. CHC chemistry, however, changes with the removal and addition of workers, probably inducing either a change in the transfer of different hydrocarbon classes or in their biosynthesis. Finally, our study emphasises intra-colonial differences between foragers and nurses, showing that these differences are greater than those between infected and uninfected workers, suggesting that ants are well able to identify the behavioural role of nestmates by their hydrocarbon profile.

Author contributions

SB, SF and FM designed the experiment. SB collected the data. SB, SF and FM analysed the data. SB wrote the first draft and SB, SF and FM revised the manuscript until completion.

Acknowledgements

[removed for privacy purposes]

Supplementary Material

Table S1. Collection sites and details for the three *Temnothorax nylanderi* populations. First entry in '*colonies for field*' and '*experimental*' data refers to the number of parasitized colonies. The number of unparasitized colonies is given in the second entry.

Population	Coordinates	Collected Colonies	Colonies for	
			Field	Experiment
Mainz	50°00'35.7 N, 8°10'48.6 E	145	13/10	20/60
Monastery Eberbach	50°02'33.0 N, 8°02'48.0 E	33	11/16	-/-
Rüdesheim	49°58'44.5 N, 7°55'24.2 E	13	2/2	-/-

Table S2. Results of the PERMANOVA and LME analyses on dataset *a*. We compared the hydrocarbon profiles, the mean chain length, the total amounts of CHCs, the proportion of *n*-alkanes, monomethyl alkanes and di- and trimethyl alkanes in relation to *worker type* (infected worker, nurse, forager) and *field parasitism status* (parasitized, unparasitized). Predictors and statistics that retained in the final models are given in bold. Pseudo-F values are presented for the PERMANOVA analysis and F-values for LMEs.

Variables	*Predictors*	*Pseudo-F/F*	*P*
Hydrocarbon profile *T. nylanderi* (PERMANOVA)	Worker type x Field parasitism	0.69	0.611
	Worker type	**8.97**	**0.001**
	Field parasitism	0.76	0.547
Mean chain length	Worker type x Field parasitism	0.53	0.467
(LME)	**Worker type**	**12.16**	**<0.001**
	Field parasitism	1.04	0.311
Total amounts of CHCs	Worker type x Field parasitism	0.30	0.583
(LME)	**Worker type**	**5.14**	**0.007**
	Field parasitism	1.08	0.302
Proportion of *n*-alkanes	Worker type x Field parasitism	0.51	0.475
(LME)	**Worker type**	**11.78**	**<0.001**
	Field parasitism	0.35	0.555
Proportion of monomethyl alkanes (LME)	Worker type x Field parasitism	0.01	0.907
	Worker type	**3.13**	**0.048**
	Field parasitism	0.17	0.683
Proportion of di-& trimethyl alkanes (LME)	Worker type x Field parasitism	0.87	0.354
	Worker type	**6.29**	**0.002**
	Field parasitism	1.08	0.302

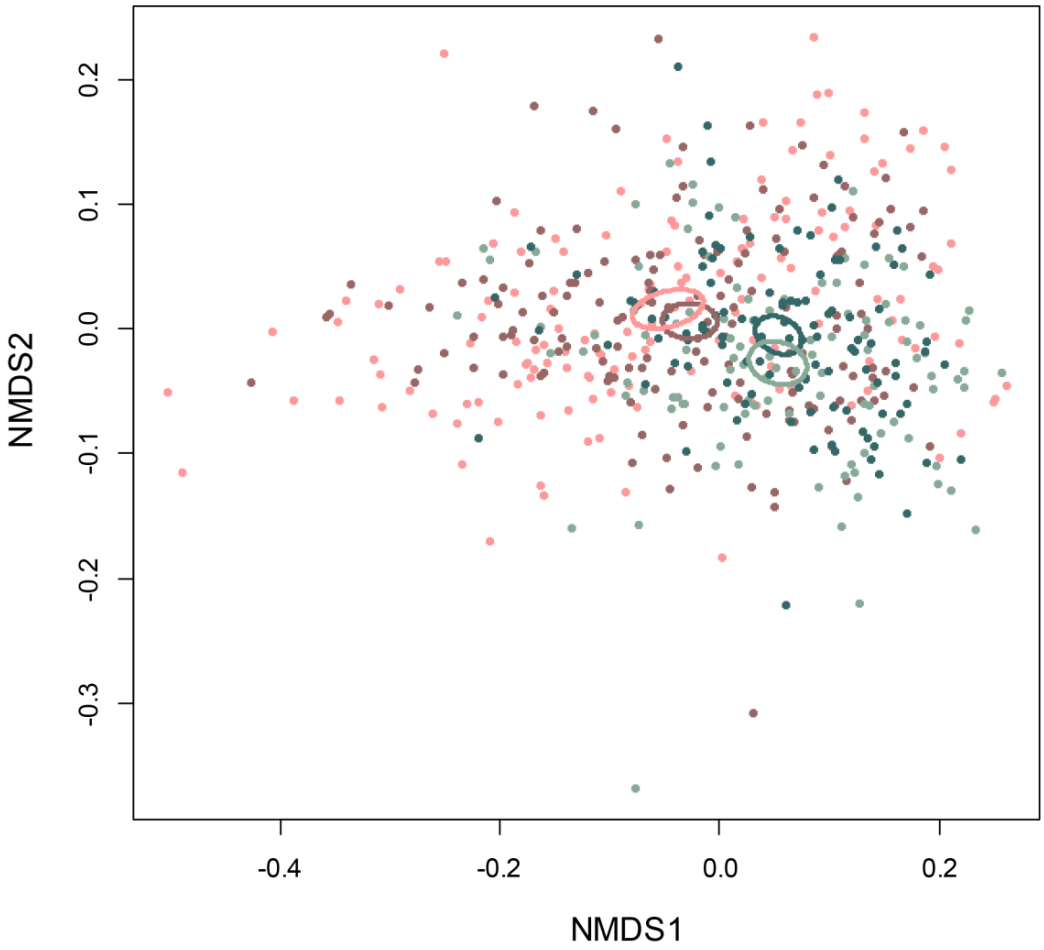

Figure S1. NMDS plot showing cuticular hydrocarbon resemblance of foragers and nurses from colonies that had nestmates removed (i.e. donor colonies = green coloured; foragers = dark green, nurse = light green) and colonies that received non-nestmates (i.e. receiver colonies; foragers = dark red, nurse = light red). Resemblance is based on the Bray-Curtis Similarity and stress value is 0.14. Ellipses represent 95% of confidence intervals.

Table S3. Results of PERMANOVA and LME analyses of dataset *b*. We compared the hydrocarbon profiles, the mean chain length, the total amounts of CHCs, the proportion of *n*-alkanes, monomethyl alkanes and di- and trimethyl alkanes in relation to *behavioral caste* (nurse, forage), *experimental treatment* (donor, receiver) and *exchanged worker type* (infected, uninfected). Predictors and statistics that retained in the final models are given in bold. Pseudo-F values are presented for the PERMANOVA analysis and F-values for LMEs.

Variables	*Predictors*	*Pseudo-F/F*	*P*
Hydrocarbon profile *T. nylanderi* (PERMANOVA)	Behavioral caste x Experimental treatment x Exchanged worker type	1.12	0.298
	Behavioral caste x Experimental treatment	**2.98**	**0.021**
	Behavioral caste x Exchanged worker type	0.79	0.537
	Experimental treatment x Exchanged worker type	1.00	0.361
	Behavioral caste	**20.98**	**<0.001**
	Experimental treatment	**9.74**	**0.002**
	Exchanged worker type	2.26	0.054
Mean chain length	Behavioral caste x Experimental treatment x Exchanged worker type	0.02	0.962
(LME)	Behavioral caste x Experimental treatment	1.50	0.220
	Behavioral caste x Exchanged worker type	0.63	0.426
	Experimental treatment x Exchanged worker type	1.80	0.180
	Behavioral caste	**4.97**	**0.026**
	Experimental treatment	**27.10**	**<0.001**
	Exchanged worker type	**4.19**	**0.041**
Absolute amounts of CHCs	Behavioral caste x Experimental treatment x Exchanged worker type	0.43	0.510
(LME)	Behavioral caste x Experimental treatment	1.07	0.301
	Behavioral caste x Exchanged worker type	0.49	0.482
	Experimental treatment x Exchanged worker type	0.10	0.755
	Behavioral caste	3.76	0.053
	Experimental treatment	**4.50**	**0.033**
	Exchanged worker type	1.03	0.310
Proportion of *n*-alkanes	Behavioral caste x Experimental treatment x Exchanged worker type	0.06	0.809
(LME)	Behavioral caste x Experimental treatment	0.33	0.568
	Behavioral caste x Exchanged worker type	0.19	0.662
	Experimental treatment x Exchanged worker type	0.73	0.392
	Behavioral caste	2.35	0.125
	Experimental treatment	**8.12**	**0.004**
	Exchanged worker type	2.04	0.153
Proportion of monomethyl alkanes (LME)	Behavioral caste x Experimental treatment x Exchanged worker type	1.22	0.269
	Behavioral caste x Experimental treatment	**7.80**	**0.005**
	Behavioral caste x Exchanged worker type	0.59	0.443
	Experimental treatment x Exchanged worker type	0.03	0.854
	Behavioral caste	**53.44**	**<0.001**
	Experimental treatment	**12.17**	**<0.001**
	Exchanged worker type	1.15	0.284
Proportion of di-& trimethyl alkanes (LME)	Behavioral caste x Experimental treatment x Exchanged worker type	0.45	0.503
	Behavioral caste x Experimental treatment	**5.56**	**0.018**
	Behavioral caste x Exchanged worker type	0.56	0.453
	Experimental treatment x Exchanged worker type	1.27	0.260
	Behavioral caste	**6.55**	**0.011**
	Experimental treatment	**25.58**	**<0.001**
	Exchanged worker type	**5.25**	**0.022**

Chapter 3

Gene expression patterns underlying parasite-induced alterations in host behaviour and life history

Barbara Feldmeyer, Johanna Mazur, **Sara Beros**, Hannes Lerp, Harald Binder and Susanne Foitzik

Based on:
Molecular Ecology, 25, 648-660

Abstract

Many parasites manipulate their hosts' phenotype. In particular, parasites with complex life cycles take control of their intermediate hosts' behaviour and life history to increase transmission to their definitive host. The proximate mechanisms underlying these parasite-induced alterations are poorly understood. The tapeworm *Anomotaenia brevis* affects the behaviour, life history and morphology of parasitized *Temnothorax nylanderi* ants and indirectly of their unparasitized nestmates. To gain insights on how parasites alter host phenotypes, we contrast brain gene expression patterns of *T. nylanderi* workers parasitized with the tapeworm, their unparasitized nestmates and unparasitized workers from unparasitized colonies. Over 400 differentially expressed genes between the three groups were identified, with most uniquely expressed genes detected in parasitized workers. Among these are genes that can be linked to the increased lifespan of parasitized workers. Furthermore, many muscle (functionality) genes are downregulated in these workers, potentially causing the observed muscular deformations and their inactive behaviour. Alterations in lifespan and activity could be adaptive for the parasite by increasing the likelihood that infected workers residing in acorns are eaten by their definitive host, a woodpecker. Our transcriptome analysis reveals numerous gene expression changes in parasitized workers and their uninfected nestmates and indicates possible routes of parasite manipulation. Although causality still needs to be established, parasite-induced alterations in lifespan and host behaviour appear to be partly explained by morphological muscle atrophy instead of central nervous system interference, which is often the core of behavioural regulation. Results of this study will shed light upon the molecular basis of antagonistic species interactions.

Keywords: behavioural manipulation, extended phenotype, host–parasite interactions, insects, transcriptome

Introduction

Parasites can manipulate the behaviour, morphology, physiology and life history of their hosts (Poulin & Thomas 1999; Thomas et al. 2010). This is particularly true for parasites with complex life cycles that are known to take control over the behaviour of their intermediate hosts in order to increase transmission to the definitive host. These host manipulations can range from slight changes in pre-existing traits to the display of entirely novel behaviours (Poulin 1994; Thomas et al. 2002), a phenomenon also called the 'extended phenotype' of a parasite (Dawkins 1982). Classical examples of parasite-manipulated host behaviour include ants that climb to the apex of grasses and remain there, facilitating their parasites' transmission to grazers such as sheep (Hohorst & Graefe 1961; Carney 1969; Libersat et al. 2009), or terrestrial insects that 'commit suicide' by jumping into water where the parasite can complete its life cycle (Moore 1995; Biron et al. 2005). The proximate mechanism of these fascinating behavioural manipulations is still poorly understood (Thomas et al. 2010; Biron et al. 2013; de Bekker et al. 2014) and is a hot topic in evolutionary biology (Van Houte et al. 2013).

The various mechanisms of parasite manipulation of host behaviour most likely evolved in the context of manipulation of other host traits required for the parasite's survival, especially the immune system (Adamo 2013). To manipulate their hosts, parasites may use a suite of different strategies: (*i*) destruction of sensory structures and muscles (Beckage 1997), (*ii*) genomic and proteomic alterations, (*iii*) psychoneuroimmunological and (*iv*) neuropharmacological mechanisms (Adamo 2013). The tight connection between the immune and the nervous system facilitates carry-over effects. Behavioural changes might thus be due to neuronal consequences of parasites addressing the hosts' immune system (Dantzer et al. 2008; Adamo 2013). Moreover, immune responses to parasite infection can have a suite of indirect effects on host phenotype, like reduced fertility, changes in melanization and increased lifespan, often based on resource trade-offs (Moret & Schmid-Hempel 2000; Ahmed et al. 2002). Parasite-induced alterations in host behaviour are expected to have a molecular basis in the central nervous system, which processes sensory information and transforms this into muscle activity (Biron et al. 2013). In some host–parasite systems, the parasite excretes neuro-effectors that directly induce behavioural changes in the host (Hakimi & Cannella 2011; McDonough & Rodriguez 2012). In other cases, parasites induce the host to secrete neuromodulators itself (Reilly et al. 1992), which is more effective for the parasite as it outsources the production costs to the host (Adamo 2013). Host–parasite genotype interactions seem to play an important role in host gene expression response upon infection and can be genotype specific (Barribeau et al. 2014).

Many manipulative parasites are known to exploit ants as intermediate hosts (e.g. Carney 1969; Moore 1995; Yanoviak et al. 2008). This is not only due to the ubiquity of ants, but also their social life style increases transmission probabilities of the parasite and thus makes them attractive targets. When parasites exploit social insects, they can influence not only their direct hosts, but also the entire society (Cremer et al. 2007; Konrad et al. 2012a). This also holds true for our focal parasite–host association with the parasitic tapeworm *Anomotaenia brevis* (Plateaux 1972; Scharf et al. 2012b) and its intermediate host, the ant *Temnothorax nylanderi*. To complete its life cycle A. brevis relies on predation of its intermediate host by its definitive hosts, the woodpeckers *Dendrocopos major* and *Dendrocopos minor* (Trabalon et al. 2000), (**Figure 1**). Ants become infected during the larval stage by being fed with bird faeces containing the tapeworm eggs (Trabalon et al. 2000). These eggs develop into larvae, penetrate the ants' gut wall and enter the haemocoel, where they transform into cysticercoids. The emerging infected adult ants exhibit a less pigmented, soft cuticle and are smaller than their uninfected brown nestmates (**Figure 11a**; Trabalon et al. 2000; Scharf et al. 2012b). Moreover, these workers are mainly inactive and remain in the nest, even when disturbed (Beros et al. 2015, Chapter 1) - a behaviour that could increase parasite transmission to the definitive woodpecker host, which is known to feed on wood-boring insects and acorns. Despite their deviant chemical profile (Trabalon et al. 2000), these parasitized workers are accepted and well cared for by their unparasitized nestmates. Indeed, they are even more often fed and survive longer than unparasitized workers (Beros et al. 2015, Chapter 1). Behavioural changes extend to all workers from parasitized colonies. Unparasitized workers in parasitized colonies showed behavioural patterns intermediate to those of parasitized workers and unparasitized workers from unparasitized colonies (Scharf et al. 2012b). Interestingly, these unparasitized nestmates suffer from a reduced survivorship (Beros et al. 2015, Chapter 1). Experimental manipulations further demonstrated that the presence of parasitized workers lowers the aggression of unparasitized workers towards intruders, indicating that parasites exploiting social animals cannot only induce phenotypic changes in their direct individual hosts, but also in unparasitized group members (Beros et al. 2015, Chapter 1).

Whether these reported parasite-induced changes are due to direct parasite manipulation of the hosts' phenotype, host defences against parasite infection, or simple by-products of infection, benefitting neither parasite nor host still needs to be clarified. To gain more insights into the proximate basis of parasite-induced phenotypic changes, a first step is to understand how gene expression changes with infection status. Here, we investigate brain gene expression patterns of parasitized workers and unparasitized workers from parasitized and unparasitized nests. In particular, as we are interested in genes underlying the behavioural changes such as the inactivity of parasitized workers and the lower aggression levels of their nestmates.

Methods

Ant collection

Temnothorax nylanderi colonies were collected from July to September 2013 from three sites close to Wiesbaden, Germany [Kloster Eberbach (KLE; n = 52): N50°02.3570, E8°02.9830; Lennebergwald (LBW; n = 79): N50°00.6720, E8°10.9060; Neroberg (NB; n = 35): N50°05.9190, E8°13.9870]. Ant colonies contained one queen and on average 81 workers (range: 32–150 workers). To exclude any bias due to differences in colony size, we included an unparasitized colony of similar size (± 15 workers), for each parasitized colony. Parasitized colonies contained a mean of 12% parasitized workers (range: 4–37%) and at least ten unparasitized workers. The total number of workers per colony differed neither between replicates nor between treatments (Kruskal–Wallis tests, p = 0.90). Furthermore, the proportion and number of parasitized and unparasitized workers of parasitized colonies were not significantly different between replicates (Kruskal–Wallis tests, p = 0.85). All colonies were kept under standardized conditions (i.e. 20°C and 12 h day/night cycle) for at least 3 weeks to minimize sample site effects. The ants were fed twice weekly with honey and crickets.

Sample preparation and sequencing

To investigate the parasites' influence on gene expression, we compared three worker groups: unparasitized workers from ant colonies without any parasitized workers (UU); parasitized workers (PP, yellow, **Figure 11a**) and unparasitized workers from parasitized nests (UP, brown, **Figure 11b**). PP and UP workers were taken from the same colonies. Ant workers differ in behaviour depending on age, their position and function in the nest (Mersch et al. 2013; Pamminger et al. 2014). To control for these behavioural differences, we selected only inside, brood care workers, which are normally the youngest workers in ant colonies, and are found directly on the brood, similar to the infected workers. Please note that after the onset of this project, it was demonstrated for this host–parasite system that worker survivorship varies with parasitism status and that infected workers survive longer (Beros et al. 2015, Chapter 1). Thus, our sample of infected workers likely contained older individuals compared to the young brood care workers. The interpretation of any expression differences, especially of longevity genes, should take these presumably parasite-induced age differences between worker groups into account.

We extracted total RNA from ant brains for two reasons: (*i*) we were mainly interested in the causes of behavioural differences between workers of the different parasitism states, (*ii*) to exclude contamination with tapeworm RNA. In adult ants, the tapeworm resides in its cysticercoid stage attached to the ants' intestine in the gaster (Plateaux 1972). To obtain a sufficient RNA yield, we pooled brains from 20 workers per replicate. We handled samples in batches of 10 brains to ensure RNA stability during brain preparation. Per sample, two batches were merged before RNA extraction. For each of the three worker groups, we prepared four biological

replicates, that is, in total; we dissected 240 ant brains from 40 colonies. Each replicate from each treatment contained brains of workers from five colonies. On average, four workers (range 3–6) were removed per unparasitized colony and the same number for each worker type from parasitized colonies.

Each colony was disturbed only once and all required ants were moved to a petri dish with a small, wet paper tissue for humidity. Once the ants calmed down, brain dissections started. We tried to keep handling times as short as possible with about 10 min on average. We cannot rule out gene expression changes due to stress from worker isolation and the time needed for brain dissection. However, these factors should have affected all worker groups to the same degree. We focussed on brain tissue specifically, as we were interested in the molecular basis of the altered host behaviours. The head of a single worker was fixated in Flexaponal® dental wax (Dentaurum, Ispringen, Germany) in a new, sterile petri dish, which was cooled on ice during the entire procedure. Under a stereomicroscope, the head was cut off with a sterile lancet and the torso moved to a second petri dish. The gaster was dissected to check for the occurrence of cysticercoids of *Anomotaenia brevis*. All workers with the yellow, parasitized phenotype were confirmed to contain cysticercoids. In two cases, workers with unparasitized phenotypes were found to be infested by the parasite, which occasionally happens in parasitized nests as noted before (Scharf et al. 2012b); these individuals were excluded from further analyses. The number of cysticercoids of dissected ants was counted, and ovary development was quantified. *Temnothorax* workers normally have two ovarioles, but no spermatheca; hence, they can only produce male offspring, which they only do in the absence of the queen (Konrad et al. 2012b). Workers were categorized either as (*i*) fertile, if we detected eggs in development in the ovarioles, (*ii*) infertile, when the ovaries clearly contained no eggs or (*iii*) undetermined, when the ovaries were undeveloped or destroyed by dissection. The latter individuals were removed from the further fertility analyses. In total, we successfully dissected the ovaries of 116 workers.

For brain dissection, the head was opened with a cut between the eyes and antennae. The brain was removed using a barbed needle and transferred into a tube containing 75µl TRIzol (Life Technologies). After ten brain dissections, the brains were homogenized and stored at -80°C. For RNA isolation of each replicate, two batches with 10 brains each were merged. Then, 150µl chloroform was added to the tube and the mixture was vigorously shaken for 5 and 15 min centrifuged at 4°C at 11 500g. The upper aqueous phase was transferred to RNase-free microcentrifuge tubes, and RNA was precipitated by adding 60µl absolute ethanol. For subsequent RNA isolation, we used the RNeasy Mini Kit (Qiagen) and followed manufacturers' instructions. Isolated total RNA was eluted in 30µl RNA-free water and stored at -80°C. Libraries were constructed at GENterprise GmbH Mainz following the standard Illumina protocol, and each library was individually tagged. All 12 libraries were pooled and sequenced with 100 bp paired-end on 1.5 lanes of an Illumina HiSeq 2500.

Adapter remains were removed using the CLC WORKBENCH v 5.1.0 (CLC bio), read quality was trimmed with an in-house script (Phred > 20; read length > 60 bp), and read quality was checked using FASTQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/).

De novo assembly and expression analyses

The trimmed reads from all 12 replicates were used for the de novo assembly of the reference transcriptome as follows. First, subassemblies based on reads from the four libraries for each of the three worker groups were generated with the CLC WORKBENCH v5.1.0 (CLC bio) with word sizes 15, 30, 45 and 60 and bubble size of 50. Based on contig summary statistics (e.g. number of contigs, average contig length), we decided to use contigs of word size 45 from all three worker-type subassemblies for a subsequent meta-assembly with MIRA (Chevreux et al. 1999) (settings: job = de novo, genome, accurate, sanger). These contigs were used as reference for the subsequent expression analyses in EDGER 3.4.2 (Robinson et al. 2010). Reads were aligned to the contigs using TOPHAT V2.0.10 (Trapnell et al. 2009) in combination with BOWTIE2 V2.1.0 (http://bowtie-bio.sourceforge.net/). Read counts were obtained using EXPRESS V1.5.1 (http://bio.math.berkeley.edu/eXpress/). Differentially expressed genes shared between groups, as well as uniquely expressed genes per group, were visualized using the online tool Venny 2.0 (http://bioinfogp.cnb.csic.es/tools/venny).

Functional annotation and enrichment analyses

All contigs were searched against the nonredundant arthropod protein database (NCBI, state December 2013) using BLASTX (Altschul et al. 1990). Functional annotation and enrichment analyses were performed using the Blast2Go online tool with default parameters (Conesa et al. 2005).

Cluster analysis

Genes belonging to the same gene network and function are assumed to show similar expression patterns (Langfelder & Horvath 2008). We therefore conducted a weighted gene co-expression network analysis, which clusters genes according to correlation in expression patterns using the R package WGCNA (Langfelder & Horvath 2008). This analysis was based on the 414 genes found to be differentially expressed between the three worker types. We adjusted the soft-threshold (β) values to ensure an approximate scale-free topology (Zhang & Horvath 2005) and set the minimum module size to 30 and a dynamic tree cut height to 0.2 to ensure a larger number of genes in each module to assess intramodule dynamics. Default settings were used for all other WGCNA parameters.

Behaviour, immunology and longevity candidate genes

Longevity candidate genes were obtained by literature search and the HAGR GENAGE database (http://genomics.senescence.info/download.html), which features longevity genes for humans and other model organisms. Immunology-related candidate genes were obtained from the immunodatabase (http://cegg.unige.ch/Insecta/immunodb) and Terrapon et al. (2014). Behavioural candidate genes were collected from numerous publications. (A list of all candidate genes and according references can be found in **'Table S1', Supporting information**).

Morphometric analysis

As our transcriptome analysis revealed lower expression of muscular genes in the brain of parasitized workers, we analysed by confocal microscopy the structure of the mandibular closer muscle located in the head. The mandibular closer muscle is one of the strongest ant muscles and much larger than the leg muscles, which are rather difficult to examine in 2 mm large workers. We compared workers of the parasitized phenotype (PP, n = 10) with their unparasitized nestmates (UP, n = 9) from 10 parasitized colonies. For this, ants were decapitated, the head capsule was fixed in a dental wax-coated dish and covered with a droplet of cooled ant-saline solution (127 mM NaCl, 7 mM KCl, 1.5 mM CaCl2, 0.8 mM Na2HPO4, 0.4 mM KH2PO4, 4.8 mM TES, 3.2 mM Trehalose pH 7.0), adapted by Christine Dittrich and Manuel Nagel, University of Konstanz, during dissection. We opened the head capsule by cutting a window between the eyes above the brain and removed the cuticle. To avoid muscle destruction, the brain and all glands, trachea and fat tissue were left inside the head capsule. Henceforth, heads were immediately transferred to an ice-cold fixative (4% paraformaldehyde) containing additional 4% glutaraldehyde to increase tissue autofluorescene and stored at 4°C for 4 days. No further staining was performed. Heads were then washed in a phosphate-buffered saline solution (PBS, pH 7.2, 5 x 20 min), followed by an ascending dehydration series of ethanol (50, 70, 90 and 3 x 100%; 20 min each) and finally cleared in methylsalicyclic acid (M-2047, Sigma-Aldrich Chemie GmbH, Steinheim, Germany). Heads were viewed as whole-mount preparations using a laser-scanning confocal microscope (LEICA TCS SP8; Leica Microsystems AG, Wetzlar, Germany). Confocal images were taken at a resolution of 1024 x 1024 pixels using the customized settings for background fluorescence and scanned with an optical depth of 3.12µm. Confocal image stacks were visually inspected and analysed using the free Leica LAS AF LITE v2.6.3 viewer. We used the stack profile tool to mark and calculate (report of pixel count values) the area of mandible closer muscles and intramuscular gaps. We used distinct landmarks (**Figure 14a, b**) to select comparable sections of the mandibular closer muscle. At the selected layer, this muscle contains some regular gaps, which were present in all individuals. These gaps and their area were deducted when calculating the total number of gaps and the total area of the mandibular closer muscle for all individuals.

Statistical analyses

All statistical analyses were performed in the R v. 2.15.2. (R Core Team 2012). Chi-square tests were performed to test for differences in the frequency of fertile individuals between parasitized and unparasitized workers, and Wilcoxon tests to test for differences in the total number of gaps and total area of the mandibular closer muscle. Gene expression differences between the three worker types were analysed using the R package EDGER V3.4.2 (Robinson et al. 2010). Genes with significant expression differences after FDR-correction (p-FDR ≤ 0.05) (Benjamni et al. 1995) were classified as differentially expressed.

Results

In total, we obtained 442 808 496 raw reads after sequencing, of which 361 828 351 remained after quality trimming. The three CLC subassemblies resulted in 56 038 – 62 047 contigs with an average contig length of 757 bp (detailed summary statistics on reads and subassemblies can be found in '**Table S2', Supporting information**). The subsequent MIRA meta-assembly resulted in 34 934 contigs, with a remaining un-assembled 14 275 original CLC-contigs ('debris'). As the CLC subassemblies were context specific, these remaining CLC-contigs are assumed to contain valuable information for each of the three worker states. We thus added the 'debris' to the MIRA contigs, resulting in a total of 49 209 contigs, with an average length of 1376 bp and a N50 of 2643 bp. A BLASTX of these contigs vs. the nonredundant protein database (December 2013) gave 18 418 hits with $<e^{-5}$, of which 11 517 were single gene hits. Of the Blast hits, 65% were found in ants followed by 26% in other hymenopteran species (data not shown).

Gene expression analyses and functional enrichment

The expression analyses revealed a total of 414 differentially expressed genes (FDR ≤ 0.05) in pairwise comparisons between the three worker types (**'Table S3', Supporting information**). In total, we identified 198 genes to be overexpressed in parasitized workers (PP) compared to the other two worker types, of which 177 were uniquely overexpressed in parasitized workers uniquely expressed (**Figure 11b**). Unparasitized workers from unparasitized colonies (UU) had 168 overexpressed genes and 73 uniquely expressed genes. Unparasitized workers from parasitized colonies (UP) had the smallest number of overexpressed genes (156) in pairwise comparisons, as well as the smallest number of uniquely expressed genes (56). These workers also shared the highest number of genes (87) with the UU workers and 13 genes with the PP workers. UU and PP workers had eight overexpressed genes in common (**Figure 11b**). Within the set of shared genes among the two unparasitized worker types, we identified the *cuticular protein 14 precursor*, which could be involved in the hardening and pigmentation of the cuticle of unparasitized individuals. Moreover, we identified three enzymes (*Esterase E4,*

Esterase FE4 and Fatty acyl-CoA reductase 2) among the uniquely expressed genes of PP workers, which might be involved in cuticular hydrocarbon synthesis (Blomquist & Bagnères 2010), potentially explaining chemical profile changes with parasitism status (Trabalon et al. 2000). The functional enrichment analyses revealed the functions 'ribosome', 'structural constituent of ribosome' and 'translation' to be the only overrepresented functional categories in PP workers in comparison with the shared overexpressed genes between the two unparasitized worker types (**Figure 12**). In the unparasitized worker types, several metabolic functions as well as actin binding was overrepresented in comparison with the uniquely expressed gene set of parasitized workers. Enrichment analyses based on uniquely expressed genes of the unparasitized worker types and the other sets with shared genes did not lead to the identification of additional overrepresented functional categories.

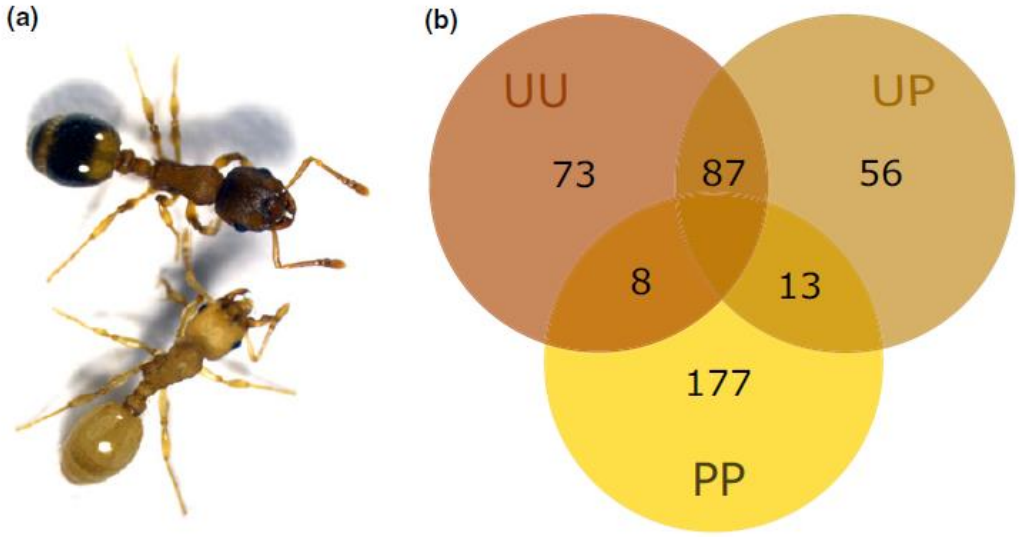

Figure 11. (**a**) *Temnothorax nylanderi* worker parasitized with the tapeworm *Anomotaenia brevis* (bottom), and its unparasitized nestmate (top). (**b**) Venn diagram depicting the patterns of unique and shared differentially expressed genes (upregulated in pairwise comparisons) among the three worker types, parasitized workers (PP) unparasitized workers from parasitized (UP) and unparasitized nests (UU).

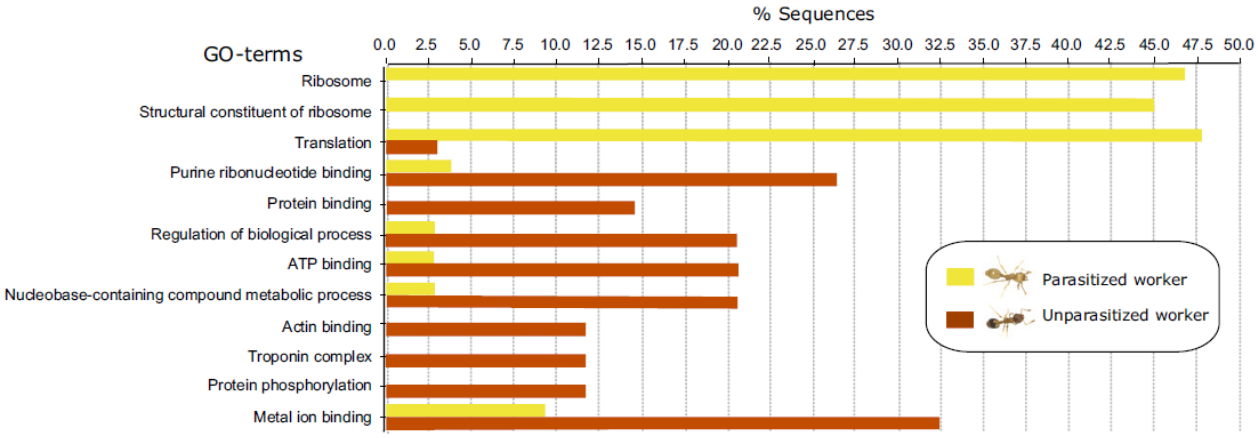

Figure 12. Functional categories overrepresented in the uniquely expressed gene set of parasitized workers vs. the shared overexpressed genes of the two unparasitized worker types (most specific terms are depicted).

Candidate genes

We identified 36 immune candidate genes in our data of the 175 genes obtained from the immunology database, none of which was differentially expressed between the three worker types. The absence of differently expressed immune genes might be due to our focus on brain tissue; however, immunity genes have been found to be differently expressed in brains of honeybee workers and queens (Grozinger et al. 2007). Comparable expression levels between the three worker types might suggest that the immune system of parasitized workers is neither impaired nor activated through tapeworm infection. The same holds for genes related to aggressive behaviour. Here, 11 of a data set of 87 could be identified, but none of them was differentially expressed. Within the 857 genes spanning data set of longevity candidate genes, 119 were expressed in the brain of *Temnothorax*-workers and two of these were differentially expressed between worker types: *Cytochrome C* was downregulated in parasitized workers in comparison with both unparasitized worker types, and *Tropomyosine* was downregulated in PP workers in comparison with UU workers. Of six genes, which were shown to play a role in parasite-mediated host behaviour (Van Houte et al.2013), the gene *Tachykinin* could be identified as being overexpressed in UP workers in comparison with PP workers. Of 172 genes upregulated in fungus-infected workers vs. control workers (de Bekker et al. 2015), 21 could be identified in our contig list. Of these, the gene *Transferrin* was upregulated in PP workers. Moreover, the genes *Actin* and again *Tropomyosin* were upregulated in unparasitized vs. parasitized workers in both studies. Expression levels of all mentioned candidate genes are shown in **'Table S4'** (**Supporting information**).

Cluster analysis

Weighted gene co-expression network analysis (WGCNA); (Langfelder & Horvath 2008) was used to identify modules (clusters) of correlated transcripts. This analysis not only allows to make inferences on genes associated with the three worker types (similar to the expression analysis), but it also associates contigs without annotation to a broad functionality as co-expressed genes are assumed to share the same function. The analysis resulted in five modules (**Figure 13; 'Table S5', Supporting information**), which are associated with one of the three worker types. Three modules (yellow, blue and brown) are positively correlated with parasitized individuals. An enrichment analysis of the corresponding contigs falling in these modules identified the functions 'ribosome', 'structural constituent of ribosome' and 'translation' in all three modules plus 'ribosome assembly' as well as 'small ribosomal subunit' in the brown and yellow module, respectively. The turquoise module, which is negatively correlated with the parasitized worker type, had the functions 'troponin complex', 'muscle contraction' and 'actin binding' enriched. No enriched functions could be detected for the grey module, which is highly positively correlated with the unparasitized workers from parasitized colonies. Only 47% of the contigs falling into this module had annotations in contrast to 67–88% in the other modules.

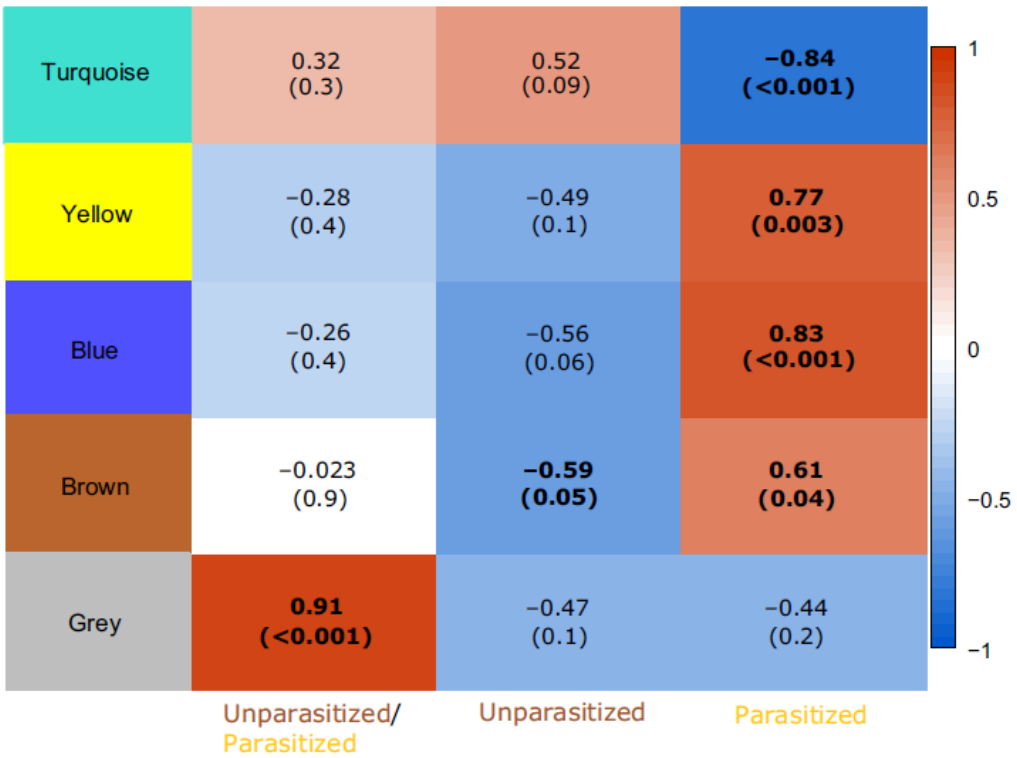

Figure 13. Module trait relationships of differentially expressed genes between the three different worker types. Rows correspond to modules and columns to worker types (Upper value: r = correlations of the corresponding module eigengenes by worker type; corresponding P-values printed below in parentheses).

Worker fertility

Tapeworm infection was associated with a lower worker fertility; 36 and 37 unparasitized workers (81% and 77%) from parasitized and unparasitized nests had eggs in development in their ovarioles, whereas only 6 (25%) of the parasitized workers (chi-square tests: overall: v2 = 26.1; $p < 0.001$; UU - PP: v2 = 21.2; p = <0.001, UP - PP: v2 = 18.0; $p < 0.001$). There was no difference in fertility between the two unparasitized worker types (UU - UP: v2 = 0.3; $p = 0.58$).

Muscle structure

Tapeworm infection was associated with a degradation of the mandible closer muscle. In comparison with their unparasitized nestmates, the mandible closer muscles of parasitized workers were less densely packed (**Figure 14a, b**). Parasitized ants had less muscle tissue (Wilcoxon test: $W = 18$, $p = 0.003$, **Figure 14c**) and more intramuscular gaps between muscle fibres (Wilcoxon test: $W = 84$; $p = 0.002$, **Figure 14d**).

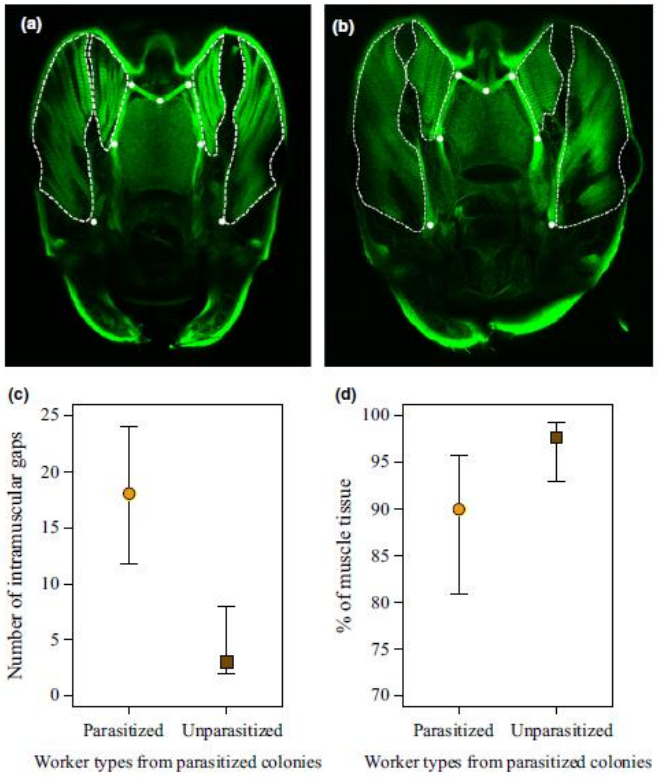

Figure 14. Confocal microscopic image of the head with morphometric landmarks and the area of the mandibular closer muscle marked in dashed lines of (**a**) a tapeworm- infested *T. nylanderi* worker and (**b**) its unparasitized nestmate. (**c**) Comparison between parasitized and unparasitized workers in the number of intramuscular gaps in the mandibular closer muscle and (**d**) the percentage of muscular tissue within the defined area of the mandibular closer muscle. Median and 75% quartiles are given.

Discussion

Parasites often induce changes in their hosts' phenotype, which can be due to host defences, by-products of infection, or parasite manipulation. Most fascinating are cases in which parasites take on the role of a puppet master and actively manipulate the behaviour of their host to increase transmission (Carney 1969; Eberhard 2000). The molecular mechanisms behind these behavioural manipulations often remain obscure, although first advances have been made (Biron et al. 2005; 2013; Van Houte et al. 2013; de Bekker et al. 2014; 2015). In the ant *Temnothorax nylanderi*, a suite of behavioural, morphological, chemical and life-history traits vary with infection by the parasitic tapeworm *Anomotaenia brevis* (Trabalon et al. 2000; Scharf et al. 2012b; Beros et al. 2015, Chapter 1). The parasite not only influences its direct host, but also unparasitized nestmates, leading to complex changes in the colony phenotype (Scharf et al. 2012b; Beros et al. 2015, Chapter 1). Here we investigate brain gene expression patterns associated with parasite-induced alterations in behaviour and life history. We identified several differentially expressed genes between parasitized workers and their uninfected nestmates. Among these are genes that can be linked to the increased lifespan of parasitized workers. Furthermore, many muscle (-functionality) genes are downregulated in these workers, which might be the cause for the observed muscular deformations and their behavioural inactivity.

Muscle atrophy in parasitized individuals

How do parasites manipulate host behaviour? In some systems, parasites make use of neuropharmacological substances such as dopamine, octopamine and serotonin to alter host behaviour (Beckage 1997; Klein 2003; Adamo 2013; Hari Dass & Vyas 2014). In our tapeworm – ant system, however, the parasite seems to impair muscle development and functioning, rather than manipulate neurological pathways. Alongside with *Tropomyosin*, we found many actin and myosin genes to be downregulated in parasitized workers. The *Tropomyosin* family of actin-binding proteins plays a crucial role in the function of actin filaments by regulating the interaction between actin and myosin (Gunning et al. 2008). These filaments not only occur in muscle cells as parts of the contractile apparatus, but also in the cytoskeleton of any cell type (Gunning et al. 2008). A deficiency in *Tropomyosin* leads to weak muscles (Corbett et al. 2001), and low expression levels are generally associated with old age in mice (Bodyak et al. 2002). These traits therefore fit the phenotype of inactive parasitized workers, which are presumably older than their uninfected nest mates due to their higher survival rate. Further, we could show by confocal microscopy that indeed the structure of the mandibular closer muscle showed strong morphological aberrations in parasitized workers in comparison with unparasitized workers. A downregulation of *Actin* (de Bekker et al. 2015), in combination with extensive atrophy of the mandibular muscles was also observed in *Camponotus leonardi* infected by the fungus *Ophiocordyceps unilateralis s.l.* (Hughes et al. 2011). Parasite-induced muscle reduction in hosts is a common phenomenon

(Beckage 1997). For example, the *Trichinella* parasite induces an inflammatory response in mice comprising an activation of oxidative stress, measurable in surrounding muscle fibres (Bruschi & Chiumiento 2011). Direct damage to the abdominal muscle of the host *Gryllus rubens* is caused by feeding of a parasitoid fly larva (Adamo et al. 1995). However, in these examples parasites directly and locally affect muscle tissue by either feeding on it or through inflammatory responses. In our system the parasitic cysticercoids are attached to the hosts' gut, whereas the observed morphological and gene expression changes occur in the ants' head, out of the direct range of the parasite. Furthermore, we would not expect a downregulation of a suite of muscle associated genes, if the observed muscle degeneration would be due to lower resource availability that is resources absorbed by the parasite.

Longevity and fertility

Recent analyses show that parasitized workers show a higher survival rate than their unparasitized nestmates and might thus be older (Beros et al. 2015, Chapter 1). Their increased lifespan might either be due to parasite-induced upregulation of longevity genes or be a by-product of other alterations, for example the lower activity level of infected workers. Future analyses controlling for age will allow to disentangle cause and effect in respect to the changed expression of longevity genes in parasitized workers. Among the differentially expressed genes according to parasitism status were *Cytochrome C* and *Tropomyosin*, which are known to be involved in oxidative processes (Corbett et al. 2001; Sohal et al. 2008; Klichko et al. 2014). *Cytochrome C* was underexpressed in parasitized workers compared to both unparasitized worker types and has been shown to be associated with decreased walking speed and lifespan in *Drosophila* (Klichko et al. 2014). This fits the long-lived phenotype observed in tapeworm-infected *T. nylanderi* workers (Scharf et al. 2012b). Moreover, among the uniquely expressed genes of parasitized workers, there were two additional genes (*Phospholipid hydroperoxide glutathione peroxidase* and *Putative oxidoreductase yrbE*) associated with oxidation reduction (Holliday 2006), possibly contributing to the increased survival of parasitized workers. Among the most abundant functional categories of differentially expressed genes between parasitized and unparasitized workers were those associated with ribosomal and translational genes. In yeast, worms, fruit flies and mice, a decrease in *TOR* (*target of rapamycin*) activity leads to a lower ribosomal biogenesis and translational activity, which in turn increases lifespan (Kaeberlein et al. 2007; Hands et al. 2009; Johnson et al. 2013 and authors therein). Ribosomal activity can vary between developmental stages, cell and tissue types (Xue & Barna 2012), and different copies of ribosomal proteins or subunits take over diverse functions (Xue & Barna 2012). In contrast to the patterns described in the above-mentioned model organisms, we find ribosomal protein genes overexpressed in longer lived parasitized workers. As we did not control for age and parasitized workers survive much better (Beros et al. 2015, Chapter 1), it is plausible that parasitized workers in our study are older than unparasitized ones and therefore have higher expression

levels of ribosomal proteins. However, there are numerous genes with opposing expression patterns known in ants in comparison with other organisms. For example, an upregulation of the *foraging* gene leads to high motility in *Drosophila* larvae (rovers) (*Dmfor*; Kent et al. 2009) and to foraging behaviour in honeybees (*Amfor*; Ben-Shahar 2005), whereas in the harvester ant *Pogonomyrmex barbatus*, young callow workers are characterized by a higher *Pbfor* expression in comparison with foragers (Ingram et al. 2005). At the same time, we also have a reversal in the longevity-fecundity trade-off (Keller & Genoud 1997) in social hymenopterans where the fecund queen outlives the sterile workers by up to decades. Alternative regulation of conserved molecular pathways associated with ageing and fecundity has recently been shown for ant queens (von Wyschetzki et al. 2015). It is thus conceivable that ageing-related mechanisms and pathways are differently regulated in ant workers then in the model organisms studied to date.

We would like to stress that even if age might seem like a confounding factor here, increased longevity is parasite-induced, and thus, the older age of infected workers is also an (indirect) effect of the parasite. Furthermore, fungus-infected *Camponotus castaneus* foragers (Hughes et al. 2011) show a similar muscle atrophy phenotype compared to noninfected foragers (of similar age), indicating that the parasite-induced muscle atrophy is not necessarily age related. The increased longevity with infected workers could also be explained by resource allocation trade-off, according to which parasitized individuals invest less in fecundity and are thus longer lived. This hypothesis has been suggested in another tapeworm system, where infection by the rat tapeworm, *Hymenolepis diminuta*, leads to an increase in lifespan and lower fecundity in the female beetle host (Hurd et al. 2001). We observed the same pattern with parasitized workers having fewer eggs in their ovaries. Lower fecundity is also reflected in the expression pattern of the fertility candidate gene *vitellogenin 3*, which is down-regulated in tapeworm-infested individuals compared to unparasitized workers. *Vitellogenin* occurs in multiple copies in ants and is responsible for its original function in yolk protein production as well as for caste differentiation (Wurm et al. 2010; Feldmeyer et al. 2014; Morandin et al. 2014).

Effect on unparasitized nestmates

The gene *Tachykinin* was found to be upregulated in unparasitized workers from parasitized colonies in comparison with parasitized workers. This gene has been linked to aggressive behaviour in *Drosophila* and a range of other organisms (Pavlou et al. 2014 and authors therein). Tachykinin-related peptide (TKRP)- deficient flies display aberrant spatial orientation (Kahsai et al. 2010) and olfactory perception as well as enhanced locomotion, pointing to a role of *TKRP* in the modulation of locomotion activity in insects (Nässel & Winther 2010). *TKRP* also functions as release factor for the metabolic neuropeptide adipokinetic hormone (Nässel 2002), which has been suggested to be a prime target for parasite manipulation via host metabolic processes (Van Houte et al. 2013). It is therefore possible that the reduced aggression observed in nestmates of parasitized workers is modulated by the

expression changes of *Tachykinin*. Whether this is caused by active manipulation of the parasite, an indirect effect of contact to parasitized individuals, or rather due to increased workforce of the unparasitized nestmates needs further investigation.

Conclusions and implications

According to the extended phenotype hypothesis (Dawkins 1982), the phenotype of infected hosts can be the consequence of parasite gene(s) being expressed. The altered phenotype can either be (*i*) adaptive for the parasite, by either increasing the parasites fitness through enhanced host-to-host transmission, or increased chances of finding a mate (Van Houte et al. 2013), (*ii*) it can result from the hosts efforts to reduce fitness costs of infection, or (*iii*) simply be a pathological side effect (Thomas et al. 2010). In our study system, the observed phenotypic changes in the host, mainly the reduced activity and longevity of parasitized workers, could be adaptive for the parasite as it increases the transmission probability to the definite woodpecker host (Trabalon et al. 2000). Inactive ant workers remain in the nest and do not participate in foraging and, through increased survival rate, have a four times higher likelihood of still being alive (data from Beros et al. 2015, Chapter 1) when an acorn is eaten by a woodpecker. Even more so, as infected workers were shown to remain in their nest site even when under attack. At this moment we cannot disentangle the cause and consequence of increased survival and muscle atrophy. As we only determined the hosts' side of the story so far, future studies should aim to identify the metabolites produced and released by the parasite to determine which genes and pathways are directly targeted. A recent study on the ant-fungus system *Camponotus castaneus* and *Ophiocordyceps unilateralis sensu lato* uncovered metabolites which are excreted by the fungus (de Bekker et al. 2014). Infection by the fungus leads to changes in activity patterns, as well as muscle atrophy in parasitized foragers vs. unparasitized foragers within the same age class (Hughes et al. 2011). Together, these results suggest that muscle atrophy might be yet another possible route for parasite-induced changes in host behaviour. Future studies, including RNAi-mediated gene knockdown and/or proteomics, may elucidate the causal link between the observed changes in gene expression and the alterations in behaviour and life history of parasitized individuals. These results will shed light on the molecular basis of antagonistic species interactions.

Author contributions

BF, SF, HL, JM and HB contributed to the design of the study. HL, BF, SB and SF collected the ant colonies. HL isolated the brains and prepared the samples for sequencing. JM and BF conducted the gene expression analysis. SB took the confocal microscope images. All authors contributed to writing the manuscript.

Acknowledgements

[removed for privacy purposes]

Supplementary Material

[can be accessed using the following link: https://doi.org/10.1111/mec.13498

Chapter 4

Parasitism and queen presence interactively shape worker behaviour and fertility in an ant host

Sara Beros, Carina Enders, Florian Menzel and Susanne Foitzik

Based on:
Animal Behaviour, in print

Abstract

Parasites with complex life cycles regularly alter host traits in their own interest. In social hosts, phenotypic alterations induced by parasites can also affect uninfected group members. The tapeworm *Anomotaenia brevis* uses *Temnothorax nylanderi* ants as intermediate hosts, reducing host activity and behavioural repertoire, but increasing lifespan. Uninfected nestmates are less active and less aggressive and suffer from higher mortality. Next to parasites, the social environment, such as the queen, influences worker behaviour, reproduction and longevity. Here, we studied how tapeworm parasitism interacts with the queen to affect the behaviour and reproductive potential of ant workers. We collected naturally parasitized and unparasitized ant colonies, and experimentally removed the sole queen in half of each colony type to induce worker reproduction. We examined the behaviour and ovary development of tapeworm-infected workers, uninfected nurses and uninfected foragers from parasitized and unparasitized colonies living under queenright and queenless conditions. Remarkably, fertility induction was most pronounced in tapeworm-infected workers, which quickly responded to queen removal by developing their ovaries. The fertility of nurses, known to have the highest reproductive potential and to be in close contact with infected workers, was not reduced by tapeworm parasitism. However, their behaviour was impacted by an interaction between parasitism and queen removal: nurses from parasitized, queenless colonies became less active, whereas no behavioural changes were observed in nurses from unparasitized colonies. Behaviour and ovary development of foragers were unaffected by the presence of tapeworm-infected workers and the queen. Our findings indicate that parasitism by a tapeworm increases rather than decreases the reproductive potential of infected workers. We further show that parasites and the presence of dominant group members interactively shape fertility and behaviour of infected and healthy group members.

Keywords: parasitism, queen, social insects, worker behaviour, worker reproduction

Introduction

Parasites affect almost all organisms on earth, and their effects on hosts can be severe and complex. Owing to their social lifestyle, social Hymenoptera (ants, some bees and wasps) are attractive hosts to a great diversity of parasites (Schmid-Hempel 1998). Parasites exploit social hosts for resources (e.g. Thomas & Elmes 1998; Foitzik & Herbers 2001), reduce their reproduction and/or decrease their survival, and change their investment strategies (e.g. Shykoff & Schmid-Hempel 1991; Imhoff & Schmid-Hempel 1999; Brown et al. 2000; Scharf et al. 2012b). Manipulative parasites use social insect workers as vessels for transmission and regularly induce spectacular behavioural alterations to their own benefit (e.g. Carney 1969; Yanoviak et al. 2008; Hughes et al. 2011; Hughes et al. 2012). The negative impact of parasites on colony life has led to the evolution of sophisticated defence mechanisms including social immunity behaviours (Cremer et al. 2007). Many social insects change their behaviour when encountering parasites to avoid or limit the costs of parasitism (e.g. Cremer et al. 2007).

Next to parasites, the behaviour and reproduction of social insect workers are strongly influenced by their colony members, in particular by the queen. She signals her presence and fertility state via chemical (Leonhardt et al. 2016) and physical cues (Konrad et al. 2012b), and workers respond by remaining sterile and taking care of her brood. However, following the queen's death or loss, workers of most social insects start to develop their ovaries (e.g. Bourke 1988). A new reproductive hierarchy is established via dominance interactions and a few dominant workers start laying haploid, male-destined eggs (Cole 1981; Bourke 1988; Heinze et al. 1997). In many ants, younger intranidal workers (nurses) are more likely to be future reproductives than older extranidal workers (foragers; e.g. Monnin & Peeters 1999; Pohl et al. 2011; Kohlmeier et al. 2017). Although a few workers gain direct fitness benefits, the aggressive interactions among workers are metabolically costly (Gobin et al. 2003) and reproductive workers often reduce their altruistic behaviours (Cole 1986; Bocher et al. 2008; Tsuji et al. 2012). In the end, most queenless, formerly monogynous colonies are destined to die (Dijkstra & Boomsma 2007), with the reproductive workers making the best of a bad situation.

The behaviour and life history strategies of social insect workers are shaped by their biotic interaction partners, both by parasites and by dominant colony members. Yet, only a few studies have demonstrated an interactive effect between these factors. For instance, in the absence of the queen, workers were shown to express lower immune responses upon pathogen challenge, becoming generally more susceptible to pathogens than workers living in societies with a queen (Bocher et al. 2008; Schneider et al. 2016; Keiser et al. 2018). In addition, ant colonies reduce their performance in colony-relevant tasks after losing the queen (Keiser et al. 2018). Parasite infection and queen loss can represent similar important and sometimes detrimental stressors for social insect colonies. In some cases, especially when parasites do not spread horizontally within the colony,

parasite-challenged individuals are cared for and allowed inside the nest (e.g. Scharf et al. 2012b; Gracia et al. 2018). The presence of infected social insects and the investment in care could be costly for the society, and these costs could be more pronounced under stressful conditions such as queen loss.

Here, we examined how tapeworm parasitism interacts with the presence/absence of the queen to affect worker behaviour and reproduction in the monogynous ant *Temnothorax nylanderi*. In this small cavity-nesting ant, parasitism by the tapeworm *Anomotaenia brevis* can be prevalent in some populations, with up to 30% of the colonies being parasitized (Scharf et al. 2012b). Ants, mostly workers but occasionally also virgin queens and males, serve as intermediate hosts for the tapeworm (Scharf et al. 2012b). Ants get infected when digesting bird faeces containing infectious tapeworm eggs (Trabalon et al. 2000; Scharf et al. 2012b). These penetrate the gut and develop in the ant's haemocoel into tapeworm larvae, called cysticercoids, which are infectious for the definitive hosts, i.e. several avian species (Gabrion et al. 1976; Trabalon et al. 2000). Tapeworm-infected ants have never been observed transmitting cysticercoids to nestmates (S. Beros, pers. observation). The infection induces several phenotypic changes in the intermediate host. The most pronounced alterations are the prolonged lifespan (Beros et al. 2015, Chapter 1) and the yellow body coloration caused by less cuticle pigmentation (Trabalon et al. 2000; Scharf et al. 2012b). The behavioural repertoire of tapeworm-infected workers is limited and they resemble neither younger nurses, which specialize on brood care, nor older foragers, which perform tasks outside the nest. Tapeworm-infected workers remain inactive close to the brood, are well fed by their nestmates and rarely forage despite their old age (Scharf et al. 2012b; Beros et al. 2015, Chapter 1). As they live among their colony members, their presence strongly affects their uninfected nestmates. Uninfected workers often feed tapeworm-infected ants, but they become less active (Scharf et al. 2012b) and are less aggressive towards enemies at the colony level than conspecifics from unparasitized colonies (Beros et al. 2015, Chapter 1). In addition, they experience a higher mortality rate, so that caring for tapeworm-infected nestmates appears to be costly (Beros et al. 2015, Chapter 1).

Workers of *T. nylanderi* exclusively reproduce in the absence of the queen (Heinze et al. 1997; Heinze 2008). Hence, we induced worker reproduction by experimentally removing the queen from field-collected parasitized and unparasitized *T. nylanderi* colonies. After queen removal, we first monitored the behaviour of individual tapeworm-infected workers, uninfected nurses (hereafter 'nurses') and uninfected foragers (hereafter 'foragers') and then dissected their ovaries. Tapeworms and other endoparasites are well known for their ability not only to manipulate insect host behaviour, but also to affect important life history traits such as fecundity and longevity (e.g. Baudoin 1975; Worden et al. 2000; Hurd et al. 2001; Ebert et al. 2004; Lafferty & Kuris 2009; Giehr et al. 2017). Cysticercoids of *A. brevis* reside in the ant's abdomen close to her ovaries (Gabrion et al. 1976) and could divert resources towards themselves and reduce the reproductive potential of infected

workers. We thus assumed that tapeworms reduce the reproductive potential of their host and predicted infected workers would be unable to develop their ovaries under queenless conditions and might resemble non-reproductive foragers (e.g. Kühbandner et al. 2014). The decreased survival rate of uninfected workers from parasitized colonies indicates that they are in poorer health and may have fewer resources available (Beros et al. 2015, Chapter 1). We thus expected that nurses from parasitized, queenless colonies could invest less in the development of their ovaries and would lay fewer eggs. If dominance fights are depriving workers of energy and resources, nurses in parasitized colonies would be additionally challenged, and the reduction in brood care and a decrease in activity should be more pronounced compared to nurses from unparasitized colonies. We also examined whether foragers would compensate for the reduced work conducted by workers that start to reproduce. Despite the biotic partners, spatial organization is a key factor driving the behaviour and ovary development of social insect workers (e.g. Brunner & Heinze 2009; Mersch et al. 2013; Kuszewska et al. 2018). Tapeworm-infected workers and nurses stay inside the nest and are spatially closer to the queen than foragers. We hence expected that the former would be generally more affected by the queen than foragers.

Methods

Ethical note

Temnothorax nylanderi is a common ant species in central-western European forests. Parasitized colonies can be frequently found in the field and we did not need to infect ants artificially. We obtained collection permits from the local forestry departments (issued by forestry Wiesbaden Chausseehaus and forestry Lenneberg). Our experimental design in the laboratory included the removal of the queen in half of the experimental colonies, the labelling and behavioural observation of individual ant workers, and the dissection of workers to record ovary development. Ants were killed by freezing before dissection. All these procedures were conducted following the institutional guidelines of animal welfare at our research facility (University of Mainz, Germany).

Ant collection and experimental design

From the end of July until early September 2016, we collected naturally parasitized and unparasitized colonies of *T. nylanderi* from two forests close to our research facility (50°05’42.8’N, 8°09’55.1’E; 49°48’38.0’N, 7°52’07.9’E). Colonies reside in pre-existing cavities such as hollow acorns or rotten sticks (Heinze et al. 1996). Because nest sites rapidly decay or become limited in space, colonies are forced to split up and inhabit multiple nests (e.g. Foitzik & Heinze 1998; Stroeymeyt et al. 2017). In early summer, up to one-third of *T. nylanderi* colonies can be queenless (Foitzik & Heinze 1998). From our collection, 8% of all parasitized (n = 74) and 19% of all unparasitized colonies (n = 221) were queenless. Yet, parasitized colonies were

not less often queenless (chi-square test: $X^2_1 = 1.40$, $p = 0.24$). For the experimental set-up following a full-factorial design (parasitized/unparasitized; queen present/absent), we decided to include only queenright colonies and remove queens experimentally. This allowed us to standardize the time of the queen's absence and we could ensure we included independent nests. We selected a total of 48 colonies, 25 of which were parasitized and 23 unparasitized. As dividing colonies into queenright (QR) and queenless (QL) parts may disrupt pre-existing hierarchies (Brunner & Heinze 2009), we removed queens from complete colonies. Queens were removed from 12 parasitized and 11 unparasitized colonies, resulting in 13 queenright and 12 queenless parasitized colonies, and 12 queenright and 11 queenless unparasitized colonies.

Colonies varied in worker number (range for parasitized colonies 30–245 workers, 130 ± 60: mean ± s.d.; range for unparasitized colonies 24–198 workers, 118 ± 52: mean ± s.d.). Parasitized colonies had 2 - 34 tapeworm-infected workers, resulting in parasitism rates between 2 and 31% (10 ± 10: mean ± s.d., 8.4 ± 7.4%: mean ± s.d.). We distributed colonies with different worker numbers evenly across the four treatments to avoid any colony size-related bias (Kruskal–Wallis-test: $X^2_{39} = 35.74$, $p = 0.62$). Each experimental colony was housed in an observation nest (precut cavity covered by glass slides; 50 × 10 mm and 3 mm high) placed in a bigger plastered box, where food (pieces of crickets and honey) and water were provided twice a week throughout the experimental time.

Worker behaviour after queen removal

Aggressive interactions between *Temnothorax* workers commence within hours of the queen's absence and can last several weeks (Heinze 2008; Brunner & Heinze 2009). On day 15 after queen removal, we removed individual workers from their colonies and labelled them between the postpetiole and gaster with thin, coloured wires (ELEKTRISOLA, 0.025 mm). This enabled us to recognize and observe individual workers. The wires were shortened at the ends so they did not hinder movement. Workers were returned to their colony and allowed to adjust to the new situation for 48 h. In each colony, two nurses, two foragers and, in parasitized colonies, two tapeworm-infected workers were labelled with wire in a random order (total of 242 ants). We identified nurses ($n_{\text{unparasitized}} = 46$, $n_{\text{parasitized}} = 50$) and foragers ($n_{\text{unparasitized}} = 46$, $n_{\text{parasitized}} = 50$) by their behaviour and position at the moment of removal, and tapeworm-infected workers ($n = 50$) by the lighter coloration of their cuticle, which is a definite sign of infection (Scharf et al. 2012b). Each marked ant was observed five times a day over 4 consecutive days (day 17–20, $n_{\text{total observations}} = 20$). We grouped the observed behaviours into five categories and calculated for each category the relative frequency (i.e. number of observed behaviours within the category divided by the total number of observations): (1) inactivity, (2) walking (i.e. inside and outside the nest cavity), (3) brood care (i.e. antennating, grooming, carrying and feeding larvae), (4) nestmate care (i.e. antennating, grooming and feeding adult nestmates) and (5) sociosanitary care (i.e.

inspection of the nest cavity, guarding the nest entrance (e.g. Waddington & Rothenbuhler 1976) and self-grooming (e.g. Zhukovskaya et al. 2013)). Observed workers did not interact with the queen, did not exhibit aggression and were not attacked by colony members. Note that behavioural observations could not be conducted entirely blind, as the presence/absence of tapeworm-infected workers and the queen were apparent to the observer. Sometimes workers could not be found during a scan but reappeared in the next scan. For 10% of the workers (24 of 242), we conducted only 14-19 observations. We kept data from these workers in the statistical analyses, as data were transformed to relative frequencies. Two foragers from parasitized colonies died during the observations and were excluded from further analysis. Although *Temnothorax* species lack distinct morphological worker castes and can be flexible in behaviour (Robinson et al. 2009), many show behavioural consistency and task specialization (Dornhaus et al. 2009; Pinter-Wollman et al. 2012). We tested whether, based on a single observation, intranidal and extranidal workers of *T. nylanderi,* here categorized as nurses and foragers, were indeed specializing in brood care and foraging, respectively. Our analyses confirmed that workers removed from inside the nest conducted more brood care (GLMM: X^2_1 = 7.26, $p < 0.0001$), while those taken from outside consequently foraged more (GLMM: $X^2_1 = 4.22$, $p < 0.0001$).

Worker reproduction after queen removal

After the last scan, we counted the eggs in all experimental colonies. We then removed all marked ants from half of the colonies of each treatment to inspect their ovary development. Workers were frozen at -20°C and ovaries were carefully dissected under a stereomicroscope (Leica Microsystems). Workers of *Temnothorax* usually possess two ovarioles (one per ovary; Alloway 1982). We did not detect any developed ovaries in 35 of 188 dissected ants (29.7%). The highest number of sterile workers was found among foragers (41.3%), followed by 22.92% among nurses and 20.83% among tapeworm-infected workers, but with no clear differences between all three (chi-square test: $X^2_2 = 4.93$, $p = 0.09$). Either the ovaries were too reduced to be detected or the fragile ovarioles were destroyed during dissection. However, as we focused on the number of oocytes in development, we kept these individuals with no ovarioles in the final analyses, with numbers set to zero for the oocytes. We checked all dissected ants for cysticercoids. Tapeworm-infected workers had at least one and up to 32 cysticercoids of *A. brevis* (mean = 8), while nurses and foragers had no tapeworm larvae.

Statistical analyses

The following analyses were all performed in R v 2.15.2 (R Core Team 2012). We constructed comprehensive models and applied stepwise removal of non-significant interactions if necessary ($\alpha = 0.05$). Colony identity was continuously entered as a random factor to account for the repeated observations of multiple individuals from single colonies in all generalized linear mixed models (GLMMs). We assessed

whether and how tapeworm parasitism and the queen influence the different worker types in parasitized and unparasitized colonies in each of the five behavioural categories. We used GLMMs following binomial distribution with a logit-link function and performed three sets of models. In the first set, we analysed exclusively tapeworm-infected workers. In five single models, the relative frequency of each behavioural category was fitted as the dependent variable and 'queen presence (yes/no)' was entered as a fixed predictor. In the second and third set of models, only behavioural data of nurses and foragers were examined. The model included 'colony parasitism (parasitized/unparasitized)' in interaction with 'queen presence (yes/no)' as fixed predictors. We further tested whether and how tapeworm parasitism and the queen affected the number of oocytes using GLMMs following a Poisson distribution with logit-link function and separately analysed tapeworm-infected workers, nurses and foragers. In the model analysing tapeworm-infected workers 'queen presence (yes/no)' served as the only fixed predictor and the number of cysticercoids as a cofactor. The models for nurses and foragers each included 'colony parasitism (parasitized/unparasitized)' in interaction with 'queen presence (yes/no)' as fixed predictors.

Additionally, we assessed the impact of tapeworm parasitism and the queen on the number of present eggs in the colonies and on the proportion of fertile workers (i.e. having oocytes yes/no). The number of present eggs was tested in a quasi-Poisson generalized linear model (GLM) with 'colony parasitism (parasitized/unparasitized)' in interaction with 'queen presence (yes/no)' as fixed predictors, and colony size as a cofactor. The latter had no effect (GLM: $LR_1 = 0.02$, $p = 0.88$). The proportion of fertile workers was analysed using a binomial GLMM with 'colony parasitism (parasitized/unparasitized)' and 'queen presence (yes/no)' in interaction. To investigate whether the prevalence of tapeworm-infected workers affected behaviour and reproduction, we repeated all analyses including the 'parasitism rate (i.e. percentage of tapeworm-infected workers)', instead of 'colony parasitism (parasitized/unparasitized)'. However, we did not find any effects of parasitism rate beyond colony parasitism (all $p < 0.15$).

Results

Effect of tapeworm parasitism and queen on worker behaviour

Tapeworm-infected workers did not alter their behaviours following queen removal (**Table 2, Figure 15a-e**). The behaviour of nurses, however, was strongly influenced by an interaction between tapeworm parasitism and queen presence or absence (**Table 2**, **Figure 15f-j**). Following queen removal, nurses from parasitized colonies were less active (**Figure 15f**) and walked less than nurses from unparasitized, queenless colonies (**Figure 15g**). In particular, the inactivity of nurses in parasitized colonies was much higher under queenless than under queenright conditions, while nurse inactivity of unparasitized colonies was similar under queenless conditions. Brood care behaviour of nurses was not affected by the presence or absence of the

tapeworm-infected workers or by the presence or absence of the queen, or an interaction between both factors (**Table 2**, **Figure 15h**). Nurses from parasitized, queenright colonies performed more nestmate care than nurses from unparasitized, queenright colonies: following queen removal, only nurses from parasitized colonies reduced their care towards adult nestmates (**Figure 15i**). The presence of tapeworm-infected workers generally reduced the sociosanitary behaviours of nurses (**Table 2**, **Figure 15j**), but neither the queen's presence nor the interaction with parasitism affected these behaviours (**Table 2**). The behaviour of foragers was overall unaffected by the presence or absence of tapeworm-infected workers or of the queen, or an interaction of both factors (**Table 2**, **Figure 15k-o**); however, inactivity of foragers was influenced by a similar interaction as in nurses, with foragers from parasitized colonies being more inactive when queenless, and foragers from unparasitized colonies being more inactive when queenright (**Figure 15k**).

Effect of tapeworm parasitism and queen on worker reproduction

In contrast to our predictions, tapeworm parasitism did not reduce the reproductive potential of tapeworm-infected or uninfected workers. Remarkably, only tapeworm-infected workers responded to queen removal by developing more oocytes in their ovaries (GLMM: $X^2_1 = 6.04$, $p = 0.014$; **Figure 16 a**). Ovary development was unaffected by the number of cysticercoids inside the ant's abdomen (GLMM: $X^2_1 = 0.00$, $p = 0.99$). In contrast, neither nurses nor foragers increased their oocyte production following queen removal (GLMM $_{nurses}$: $X^2_1 = 2.74$, $p = 0.10$; GLMM $_{foragers}$: $X^2_1 = 1.13$, $p = 0.29$). Tapeworm parasitism did not negatively influence the fertility of uninfected workers, either alone (GLMM $_{nurses}$: $X^2_1 = 0.11$, $p = 0.74$; GLMM $_{foragers}$: $X^2_1 = 2.74$, $p = 0.10$) or in interaction with queen presence (GLMM $_{nurses}$: $X^2_1 = 1.15$, $P = 0.28$; GLMM $_{foragers}$: $X^2_1 = 0.03$, $p = 0.85$). Queenless nurses of parasitized colonies developed as many oocytes as queenless nurses from unparasitized colonies ($z = 1.34$, $p = 0.18$; **Figure 16 a**). The number of eggs present in the colony as well as the proportion of fertile workers was only explained by the queen presence (likelihood ratio: $LR_1 = 13.77$, $p < 0.001$; **Figure 16 b**; GLMM: $X^2_1 = 5.14$, $p = 0.02$), but not by colony parasitism (GLM: $LR_1 = 1.04$, $p = 0.31$; GLMM: $X^2_1 = 0.25$, $p = 0.62$) or an interaction between both factors (GLM: $LR_1 = 0.02$, $p = 0.88$; GLMM: $X^2_1 = 0.12$, $p = 0.73$). Hence, queenless colonies had generally more eggs present (**Figure 16 b**) and more fertile workers than queenright colonies.

Table 2. Model selection results from generalized linear mixed model (GLMM) analyses on the behavioural data.

		Tapeworm-infected workers		Nurses		Foragers	
Behaviour	Predictors	X^2_1	*p*	X^2_1	*p*	X^2_1	*p*
Inactivity	Colony parasitism	-	-	0.79	0.37	0.11	0.74
	Queen presence	1.35	0.26	**5.59**	**0.02**	0.0	0.99
	Colony parasitism * Queen presence	-	-	**10.25**	**< 0.01**	**4.14**	**0.04**
Walking	Colony parasitism	-	-	2.87	0.09	0.43	0.51
	Queen presence	0.08	0.77	1.01	0.32	0.09	0.77
	Colony parasitism * Queen presence	-	-	**10.54**	**< 0.01**	0.46	0.50
Brood care	Colony parasitism	-	-	0.74	0.39	2.00	0.16
	Queen presence	0.15	0.70	3.66	0.06	0.00	0.96
	Colony parasitism * Queen presence	-	-	0.89	0.35	3.38	0.07
Nestmate care	Colony parasitism	-	-	2.63	0.11	0.23	0.63
	Queen presence	2.43	0.12	1.43	0.23	0.07	0.79
	Colony parasitism * Queen presence	-	-	**4.77**	**0.03**	0.24	0.63
Sociosanitary care	Colony parasitism	-	-	**10.72**	**< 0.01**	0.60	0.44
	Queen presence	0.41	0.52	0.71	0.40	0.65	0.42
	Colony parasitism * Queen presence	-	-	2.91	0.09	2.98	0.08

The five behavioural categories (inactivity, walking, brood, nestmate and sociosanitary care) were analysed separately for tapeworm-infected workers, nurses and foragers. Tapeworm-infected workers were analysed in relation to 'queen presence (yes/no)', nurses and foragers in relation to 'colony parasitism (parasitized/unparasitized)' and 'queen presence (yes/no)' in interaction. Significant predictors are given in bold.

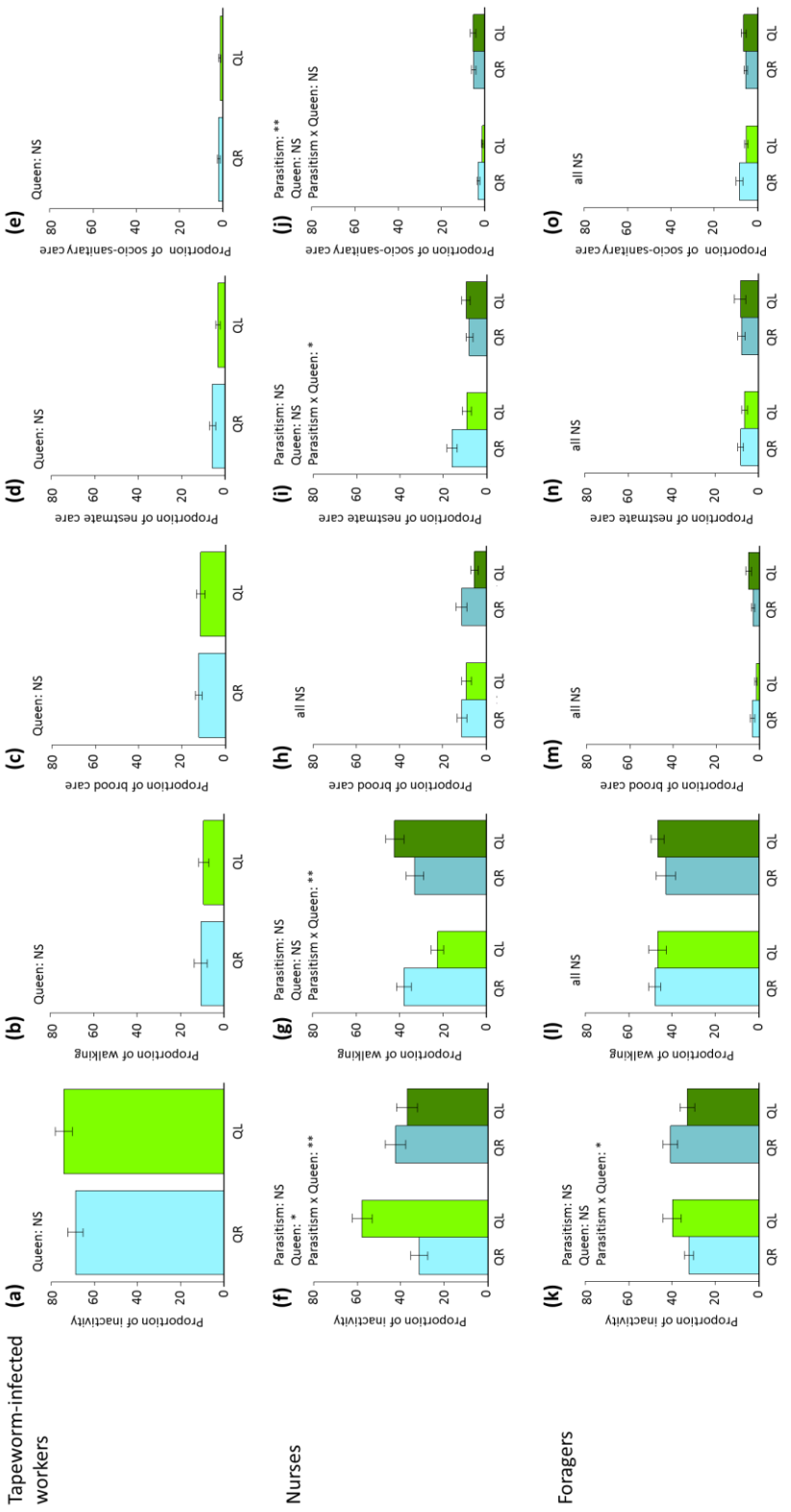

Figure 15. Differences in the five behavioural categories for (a–e) tapeworm-infected workers, (f–j) nurses and (k–o) foragers from parasitized (light) and unparasitized colonies (dark) under queenright (QR/blue) and queenless (QL/green) conditions. Vertical lines show SEs. Significant (*$p < 0.05$, **$p < 0.01$) and non-significant results of main effects and interactions are stated in the figure.

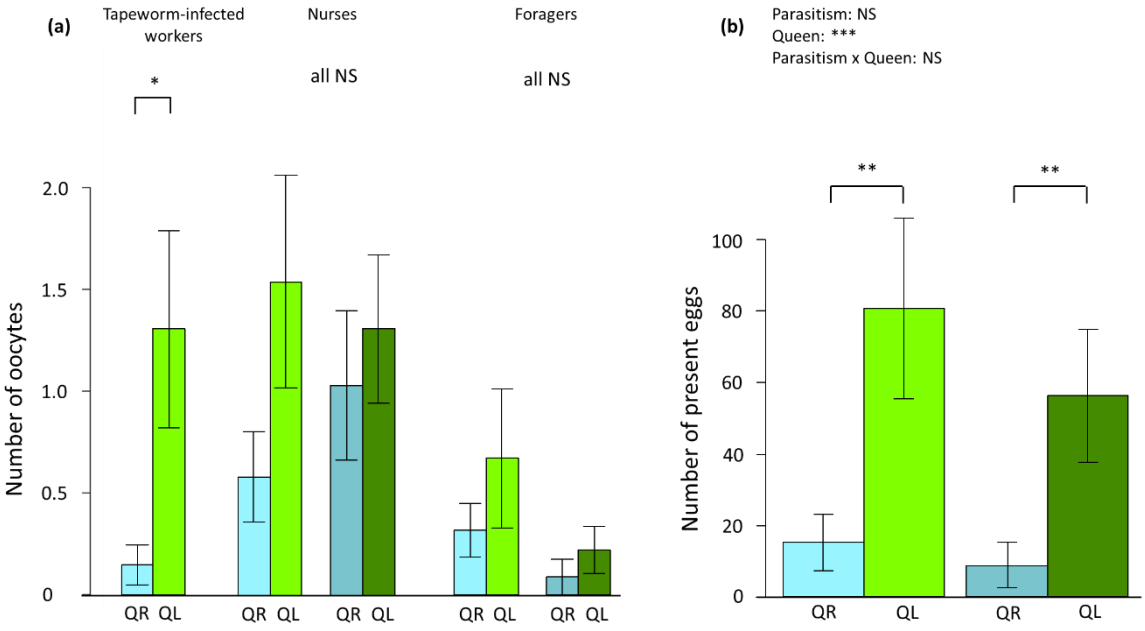

Figure 16. (**a**) Differences in the mean number of oocytes for tapeworm-infected workers, nurses and foragers in relation to colony parasitism (parasitized/light; unparasitized/dark) and queen presence (yes/QR/blue; no/QL/green). (**b**) Differences in the mean number of eggs in the colonies in relation to colony parasitism (parasitized/light; unparasitized/dark) and queen presence (yes/QR/blue; no/QL/green). Vertical lines show SEs. Significant (*p < 0.05, ** p < 0.01, *** p < 0.001) and non-significant results of main effects and interactions are stated in the figure.

Discussion

Workers from many ant species respond to the queen's absence by developing their ovaries and laying eggs. Here, we studied whether this onset of reproduction and the behavioural changes following queen removal are affected by tapeworm parasitism. Contrary to our predictions, we found that especially tapeworm-infected workers strongly developed their ovaries following queen removal. Ovary development in uninfected nestmates was unaffected by the parasitism state of the colony, but tapeworm parasitism changed the behaviour of uninfected nurses, and these effects interacted with the queen's presence. Thus, tapeworm parasitism caused behavioural changes in healthy group members, but did not affect their reproductive potential.

No reduced reproductive potential of tapeworm-infected workers

Parasitism is typically associated with costs and damage to the host. Helminth larvae use their intermediate host not only for transmission to the next host, but also as a source of nutrients.

By reallocating resources from the host to their own development and growth, endoparasite larvae can influence the trade-offs in their hosts' life history traits (e.g. Hurd et al. 2001). Our findings demonstrate that long-lived tapeworm-infected workers were able to allocate enough resources into the development of their ovaries and that this effect was further unrelated to infection intensity. Although higher parasite loads can impose higher fitness costs on their hosts (Brown et al. 2002), a few studies have found intensity-independent effects of trophically-transmitted parasites (e.g. Benesh & Valtonen 2007; Franceschi et al. 2008).

One possible explanation for the absence of negative effects on reproduction and lifespan could be the social lifestyle of the intermediate host. Because social insects can provide enough food and frequently care for infected nestmates (e.g. Scharf et al. 2012b; Gracia et al. 2018), it is likely that a better nutritional status of tapeworm-infected workers offsets the cost of resource deprivation by the parasite. Indeed, tapeworm-infected workers beg more often for food and are more often fed by their nestmates (Scharf et al. 2012b). Moreover, increased food availability might compensate for the costs of parasitism. For instance, the rat tapeworm, *Hymenolepis diminuta*, reduces the reproductive success of its intermediate beetle hosts by releasing a molecule that inhibits vitellogenin synthesis in the beetle's fat body (Warr et al. 2006). Infected beetles, however, can circumvent the negative impact on their reproduction by increasing their food intake and specifically consuming more carbohydrates (Ponton et al. 2011). In addition, caterpillars of the moth *Spodoptera littoralis* successfully fight off highly virulent pathogens when eating a protein-biased diet (Lee et al. 2006a).

The interrelation between reproduction and lifespan is reversed in social insects, in particular in ants and bees. The increased reproductive effort extends the lifespan of queens and workers (e.g. Lopez-Vaamonde et al. 2009; Heinze et al. 2013; Dixon et al. 2014; Kohlmeier et al. 2017). It is possible that the parasite-induced increase in lifespan has a positive effect on the reproductive potential of tapeworm-infected workers. If reproduction and lifespan are linked on a molecular level, the higher reproductive potential of tapeworm-infected workers might be a by-product of their extended lifespan. However, the current and a previous investigation (Feldmeyer et al. 2016, Chapter 3) show that tapeworm-infected workers have poorly developed ovaries in the presence of the queen. Thus, the higher reproductive potential is only manifested under queenless conditions. Possibly, tapeworm-infected workers are effectively policed by their nestmates (Ratnieks & Wenseleers 2005), as they are more often attacked in the presence of the queen compared to uninfected nestmates (Trabalon et al. 2000; Scharf et al. 2012b).

Tapeworm parasitism affects behaviour of nurses

Despite the reduced life expectancy in the presence of tapeworm-infected workers (Beros et al. 2015, Chapter 1), queenless nurses in parasitized colonies were able to invest as much in their ovary development as queenless nurses from unparasitized colonies. This finding, together with the results for tapeworm-infected workers, implies that parasitism by *A. brevis* has rather benign effects on the reproductive potential of workers. A negative impact was also not detectable on the colony level, as queenless, parasitized nests possessed as many eggs as queenless, unparasitized ones. Ovary dissection of nurses revealed that workers start developing eggs in the presence of the queen and independent of parasitism, and that rank orders are already formed in the queen's presence (Brunner & Heinze 2009). Eggs are probably laid by the dominant workers (Brunner & Heinze 2009). In our study, queen removal did not lead to a significant increase in ovary development, which suggests that the nurses we dissected were not among the small proportion of reproductive workers in *T. nylanderi* (Heinze 2008). This may further explain why we did not find reduced brood care behaviour, as often reported in reproductive workers (Cole 1986), or a more pronounced decline in brood care behaviour of nurses in queenless, parasitized colonies.

Although tapeworm parasitism did not affect fertility, it changed the ants' behaviour as previously shown (Scharf et al. 2012b; Beros et al. 2015, Chapter 1). Nurses of parasitized colonies behaved differently, either with or without the queen. In the presence of the queen, they provided more nestmate care and performed less sanitary behaviour than queenright nurses of unparasitized colonies. Queenless nurses in parasitized colonies became overall more inactive than queenless nurses from unparasitized colonies. High levels of inactivity may seem inefficient and have been proposed to negatively impact colony efficiency (Cole 1986). Yet, inactivity is a common phenomenon in *Temnothorax* ants (e.g. Dornhaus et al. 2008; Charbonneau et al. 2015). Inactive workers are likely to serve as food stores, are younger and replace active workers which could positively contribute to colony persistence and resilience (e.g. Hasegawa et al. 2016; Charbonneau et al. 2017). However, the reduced nestmate care in queenless parasitized colonies may affect the provision of tapeworm-infected workers and thus their survival.

Spatial organization and worker reproductive potential

The investment in aggressive interactions required to establish a new reproductive hierarchy is costly in terms of time and energy (Cole 1986; Gobin et al. 2003). Hence, age and body size are relevant factors in the hierarchy formation, but also more generally in task allocation in social insects (Robinson 1992). In many ants, including *Temnothorax*, younger and larger workers tend to have a higher reproductive potential than older and smaller workers, and frequently dominate reproduction (Pohl et al. 2011; Modlmeier et al. 2012; Kohlmeier et al. 2017). The finding that tapeworm-infected workers become fertile indicates that even the oldest and smallest workers can possess a high reproductive potential (Scharf et al.

2012b; Beros et al. n.d., Chapter 5). In fact, many of our findings can be explained by the spatial organization within the nest of *T. nylanderi*. Nurses, but also tapeworm-infected workers, are more likely to spend a significant time on the brood pile or in close contact with the queen (Scharf et al. 2012b; Kohlmeier et al. 2018a). Foragers, on the other hand, are more likely to be found in the periphery of the colony (Sendova-Franks & Franks 1995), and therefore have less contact with the queen, tapeworm-infected workers and nurses. We observed that both nurses and tapeworm-infected workers become fertile, and that nurses moreover change their behaviour. We further found no influence of the queen and tapeworm-infected workers on the fertility and behaviour of foragers. The close proximity of nurses and tapeworm-infected workers to the queen makes it very likely that these workers are the first to perceive the physical and chemical presence of the queen and respond faster to queen removal.

Conclusions

Our study reveals that parasites do not systematically reduce host fertility, and that the tapeworm *A. brevis* has benign effects on worker reproduction in the ant *T. nylanderi*. Despite their advanced age, tapeworm-infected workers respond to social cues (i.e. the queen's absence) by initiating oocyte production. These findings demonstrate that the impact of biotic interaction partners does not necessarily extend to all colony members, and that spatial proximity, rather than age and body size, can be relevant for worker reproduction and worker behaviour in *Temnothorax* ants.

Author contributions

SB, CE and SF designed the study. CE collected the data. SB analysed the data. SB wrote the first draft and SB, FM and SF revised the manuscript until completion.

Acknowledgements

[removed for privacy purposes]

Chapter 5

Extreme lifespan extension in tapeworm-infected ants facilitated by increased social care and upregulation of longevity genes

Sara Beros, Inon Scharf, Anna Lenhart, Matteo Antoine Negroni, Florian Menzel and Susanne Foitzik

Based on:
Manuscript, submitted

Abstract

Parasites of social insects present striking cases of host manipulation, but how they alter their social hosts' phenotype is poorly understood. Here, we focused on a trophically-transmitted tapeworm, *Anomotaenia brevis*, which parasitizes the ant *Temnothorax nylanderi* as an intermediate host. Our three-year study revealed that this tapeworm drastically increases the lifespan of ant workers. The mortality rate of infected workers may get as low as that of queens, which can live for decades. In contrast, uninfected nestmates demonstrated increased mortality compared to workers of unparasitized colonies. We examined the proximate mechanisms behind the increased survival and found that infected workers receive more social care than queens. Infected workers resembled young workers in their metabolic rate and lipid content, but differed in the expression of the gene *Transferrin*. *Transferrin* is known to be involved in the response to oxidative stress and we found it upregulated in both infected workers and queens. When exposed to paraquat-induced oxidative stress, *Transferrin*-dsi*RNAi* led to an increased mortality only in infected workers. We thus demonstrate an extreme lifespan extension following tapeworm infection, probably achieved through greater social care and the upregulation of protective genes, resulting in a phenotype combining aspects of young workers and old queens.

Keywords: host manipulation, social insects, host lifespan, *Transferrin*, oxidative stress, RNA interference

Introduction

Parasites are highly successful organisms comprising millions of species that vary in many aspects of their biology, such as their life cycle and exploitation strategies (Poulin & Morand 2000; Hughes & Libersat 2018). Some parasites regularly alter the phenotype of their hosts to promote their transmission or reproduction (Moore 2002; Thomas et al. 2010; Hughes et al 2012; Cézilly et al. 2013). Consequently, infected hosts can develop into deeply modified organisms showing aberrant behaviours, morphologies and physiologies (e.g. Berdoy et al. 2000; Thomas et al. 2002; Huhges et al. 2011; Kristensen et al. 2012). These parasite-induced changes in hosts are often considered manipulations by parasites in order to secure survival, or to enhance transmission or dispersal (we hereafter refer to parasites inducing such changes as manipulative parasite; (Moore 2002; Hughes et al. 2012)). For example, trophically-transmitted parasites preferably modify their intermediate hosts in such a way that these become more prone to predation, which consequently helps the parasite to complete its life cycle (Parker et al. 2003). Yet, only few experimental studies have demonstrated parasite-induced alterations to be truly beneficial for parasites (see examples in Hughes & Libersat 2018). In most other systems, the distinction between actual host manipulation, host compensatory responses, or side-effects of infection remains challenging (Herbison et al. 2018). In particular, phenotypic changes that are both multidimensional and unspecific call for more in-depth investigations (Cézilly et al. 2013). Determining the proximate causes is an essential step towards acquiring a better understanding of the pathways that underlie the phenotypic alterations, and can provide insights into the mechanisms used by parasites.

Parasites primarily exploit their hosts for resources, and thereby often impair their hosts' fitness. Increased host mortality and reduced host reproduction up to complete sterility are well-documented consequences of parasite infections (Baudoin 1975; Hurd et al. 2001), and can be used as fitness proxies to determine parasite virulence (e.g. Ebert et al. 2004). Life-history traits such as growth, reproduction and survival are traded-off against one another, because the available energy is limited, and its allocation into one trait comes at the expense of another (Stearns 1992; Agnew et al. 2000). The diversion of energy from reproduction to survival in hosts, or vice versa, depending on the parasites' life-history, is one of the suggested proximate explanations for the life-history changes observed in infected animals (Hurd 2001; Hurd et al. 2001; Vézilier et al. 2012). Infected hosts can counteract parasitic exploitation by reaching maturity earlier or by increasing their reproductive effort, shifting the balance point to current *vs.* future reproduction, as the future reproductive success becomes less certain following parasite infection (Fredensborg & Poulin 2006; Michalakis 2009, Giehr et al. 2017). By definition, parasitic manipulations reduce host fitness, for example by making hosts more vulnerable to predation (Parker et al. 2003). However, other manipulative parasites increase the lifespan of their hosts (e.g. Amat et al. 1991; Hurd et al. 2001; Dianne et al. 2011; Vézilier et al. 2012), such as by attempting to reduce predation risk of their

hosts until the parasite is ready for its next host (e.g. Dianne et al. 2011). Moreover, parasites could also extend the lifespan of their hosts in order to increase the likelihood of transmission, if predation events are generally low (Seppälä & Jokela 2008; Beros et al. 2015, Chapter 1).

Parasites of social insects, in particular ants, include some of the most striking and best-studied examples of host manipulation (Moore 2002; Hughes & Libersat 2018; de Bekker et al. 2018). The present study focused on the proximate causes for the prolonged survival of tapeworm-infected *Temnothorax nylanderi* ants, which serve the trophically-transmitted endoparasite *Anomotaenia brevis* as intermediate hosts (Beros et al. 2015, Chapter 1). This tapeworm needs to reach its final woodpecker hosts in order to reproduce sexually (Plateaux 1972; Trabalon et al. 2000). Infected workers remain predominantly inactive inside the nest and neglect certain colony tasks (Scharf et al. 2012b). They may be easy prey for woodpeckers as they show reduced escape responses (Beros et al. 2015, Chapter 1). Infected workers are more likely to survive over few weeks, but their uninfected nestmates suffer higher mortalities than uninfected non-nestmates, revealing colony-level costs of infection (Beros et al. 2015, Chapter 1). Brain gene expression varies with tapeworm infection (Feldmeyer et al. 2016, Chapter 3). Several genes associated with longevity are differently expressed in infected workers (Feldmeyer et al. 2016, Chapter 3). An upregulated gene in *A. brevis*-infected workers is *Transferrin*, which codes for a large insect glycoprotein whose primary function is to bind and transport iron essential for many metabolic processes (Nichol et al. 2002; Kucharski & Maleszka 2003; Thompson et al. 2003; Geiser & Winzerling 2012). Moreover, *Transferrin* has been implicated in vitellogenesis and immune responses of insects, and can also act as an anti-oxidant against toxic reactive oxygen species (ROS); (Yoshiga et al. 1997; Nichol et al. 2002; Valles et al. 2005; Paily et al. 2007; Lee et al. 2006b; Kim et al. 2008). Cell and tissue damages mediated by ROS can cause ageing (Finkel & Holbrook 2000). The expression of genes involved in cellular repair mechanisms is strongly elevated in social insect queens, which have exceptionally long lifespans of up to three decades (Lucas & Keller 2016; Tasaki et al. 2018). Thus, anti-oxidant genes may play a role in modulating lifespan in ants.

Our study had four aims: first, Beros et al. (2015, Chapter 1) had demonstrated that infected workers survive longer than uninfected workers. However, it remained unclear how long infected workers can fight ageing and whether their survival rates resemble more that of young uninfected workers or of queens. We therefore tracked worker and queen survival in parasitized and unparasitized colonies over three years, until over 95% of the uninfected workers and 50% of the queens had died. Secondly, we were interested in how the lifespan extension in infected workers is achieved. As parasites extract resources from their hosts, energy needs might shift. We thus analysed several physiological parameters such as metabolic rate, body mass and lipid content, which are good health indicators. As tapeworm larvae may consume a significant amount of the host ant's nutrients, we expected a higher metabolic rate and reduced lipid content in infected workers. However, *A. brevis*-

infected workers are able to develop their ovaries and start egg production following queen removal, suggesting that they do not necessarily lack resources (Beros et al. 2019, Chapter 4). Thirdly, social hosts obtain the required resources from their nestmates and this social care could compensate for the energetic costs imposed by the tapeworm (Amdam 2011). We therefore investigated how much care infected workers receive in comparison to queens and uninfected workers. Because initial evidence indicated that uninfected workers from parasitized colonies survive shorter for a shorter period of time compared to workers from unparasitized colonies, we analysed how their metabolic rate, body mass and lipid content were affected by the presence of infected workers, potentially indicating physiological stress. Finally, we sought to determine whether the upregulation of *Transferrin* might explain the improved survival of infected workers. For this purpose, we used an RNAi-mediated downregulation of *Transferrin* in combination with paraquat-induced oxidative stress to examine whether this leads to higher mortality in infected workers in comparison to uninfected workers.

Methods

Ant colony collection

All colonies were collected in forested areas in the region of Mainz-Wiesbaden, west Germany, in three different sites ((i) 50°00'36.4" N, 8°10'47.3" E; (ii) 50°02'29.4" N, 8°02'46.6" E; (iii) 50°05'42.8" N, 8°09'55.1" E).

Long-term worker and queen survival

To study the long-term direct and indirect effects of tapeworm parasitism on lifespan, we tracked the survival of individual queens and workers of different age classes from parasitized and unparasitized colonies for a period of over three years (1110 days, start: 22nd September 2014, end: 6th of October 2017). We collected 30 parasitized and 28 unparasitized *T. nylanderi* colonies in October 2013 and May 2014. All colonies were queenright and comprised between 22 and 245 workers (121 ± 58 workers: mean ± s.d.). Colony size did not differ between parasitized and unparasitized colonies (Wilcox-test: $W = 429.5$, $p = 0.89$).

Temnothorax ants have a synchronized annual reproductive cycle. During a two-week window in summer, all new workers and sexuals emerge from the pupae. We took advantage of the synchronized emergence of new workers in mid-September 2014 to identify young and old workers. Young callow workers are easily distinguishable from older ones by a light, unsclerotized cuticle. *Temnothorax nylanderi* ants are brownish with a characteristic dark abdominal stripe, which is already visible in callows, but is missing in infected workers as these individuals are completely yellow (Trabalon et al. 2000, Scharf et al. 2012b). Within a day of emergence, we wire-marked in each colony (ELEKTRISOLA, 0.025 mm) five callows ($n = 285$) and five foragers ($n = 290$). In one parasitized colony, we did not observe any new-emerged workers. All foragers were old workers, which were taken for

marking while foraging outside of the nest. Young workers in *Temnothorax* ants conduct mainly nursing duties (Kohlmeier et al. 2018b) and we therefore refer hereafter to newly emerged workers as nurses. In addition, we wire-marked one to five infected workers in parasitized colonies (n = 103). Because no emerged callow worker was infested with *A. brevis*, all infected workers were of a minimum age of one year at the onset of the observations. As *T. nylanderi* is a strictly monogynous species (Buschinger 1968), some of the queens at the onset of our observations were already a few years old, having successfully established a colony with up to 245 workers. Queens can be easily recognized from their larger size and caste-specific morphology and were therefore not marked individually.

We kept the colonies in artificial nests in boxes in climate chambers, set to temperatures and photoperiods typical to the season (December-February: 10°C : 5°C day : night (DN) temperature and 10h : 14h light : dark (LD) period; March-May: DN 20°C : 15°C and LD 12h : 12h; June-August: DN 25°C: 18°C and LD 12 h : 12 h; September-November: 18°C : 13°C and LD 12 h : 12 h). We recorded the survival of all marked workers and the queens at 10-day intervals. The day of death was the recording day on which a previously alive ant was found dead. During the three-year tracking, 16.6 % (48 of 290) of marked young workers, 20.7 % (60 of 290) of marked foragers and 13.9 % (15 of 103) marked infected workers disappeared, with no corpses found. For these individuals, the day of disappearance was entered as day of death. On each observation day (i.e. every 10 days), colonies were fed with pieces of crickets and a droplet of honey, except during hibernation (December-February), when we provided colonies with a droplet of honey at every second observation. Water was offered *ad libitum* throughout the entire observation period.

Ant survival was analysed using Cox proportional hazards regression models allowing for right censored data, that is, the number of days until death per individual, using the coxme function in R version 2.15.2 (R Core Team 2012). First, we examined whether the survival of nurses, foragers and the queens differed between parasitized and unparasitized colonies. We entered colony parasitism status (parasitized/ unparasitized) and ant category (nurse/ forager/ queen) in interaction as explanatory variables and, additionally, the colony and individual identity as random factors, because multiple individuals from the same colony and within the same category were observed. In a second model, we focused exclusively on parasitized colonies and examined survival rates of infected workers, nurses and foragers, and their queen. The model included only the ant category (infected/ nurse/ forager/ queen) as the explanatory variable. Colony and individual identity served again as random factors.

Worker and queen metabolic rate and lipid content

In April and May 2018, we collected and additional 14 parasitized and 14 unparasitized colonies. All colonies were headed by a single queen, contained 69 ± 33 workers (mean ± s.d.) and did not differ in the number of workers and brood (Wilcox-test, both $p > 0.7$). Infected workers and queens were easily recognized by

their distinct morphology. Foragers and nurses were identified by their behaviour and location: foraging outside the nest or conducting nursing duties inside the nest. We had no data on workers age, but given previous evidence, we assumed foragers to be older than nurses (see also Kohlmeier et al. 2018a).

We firstly measured O_2-consumption of single workers and queens from each parasitized and unparasitized colonies. Measurements were taken with the MicroRespiration system from UNISENSE, following their custom protocol (UNISENSE Denmark). Individual ants were isolated from their colony and were placed in the micro-respiration chamber (v = 0.448 ml), which was sealed with agar and paraffin oil. The glass chamber was transferred to a water bath at a constant temperature of 23°C. O_2-consumption was measured in the chamber lids using a thin capillary that served as oxygen microsensor. All ants were weighed directly after the measurement (accuracy of 1µg; PESCALE Wägetechnik). Real-time O_2-consumption was recorded for 10 min and viewed using the free software SensorTraceBasic v 3.0.200. We calculated the respiration rate using the linear section of the O_2-consumption slope (from minute 5 to minute 10), corrected for the body mass (mg) of individuals. The variable we used was the slope of O2 consumption (µmol/L) plotted against time (sec), divided by the ant mass (mg), multiplied by the chamber volume (ml), hereafter „metabolic rate".

After completing respiration measurements, ants were individually marked with coloured wires and returned to their colony. On the following day, we scanned the behaviour towards the focal individuals 20 times and calculated the frequency of social care (i.e. being antennated, groomed and fed). The next day, all ants were individually frozen at -20°C. To extract their lipids, they were placed in a chloroform/methanol mixture (2:1, v/v) for 24h (Folch 1957). Nonadecanoic acid (C19:0) was added as internal standard (20µl in DCM/MeOH, 2:1 v/v; 0.2 mg/ml). The extracts were then fractionated in Chromabond SiOH columns (1 ml; Macherey-Nagel). Each column was conditioned with chloroform and hexane and lipids were eluted with chloroform. The samples were dried under a nitrogen stream and dissolved in 250 µL of a 2:1 dichloromethane/methanol mixture (v/v). Lipid extracts were analysed with a 7890A gas chromatograph (Agilent) coupled to a 5975C mass-selective detector (Agilent). The oven program started at 60°C for 1 min, then increased by 15 K / min to 150°C, followed by an increase by 3 K / min to 200°C and finally a ramp of 10 K / min up to 320°C, where it remained constant for 10 min. Peak areas were integrated manually using the Agilent software MSD Chem Station E.02.02. The data were then exported to MS Excel and manually aligned. The fatty acids had chain lengths between C_{12} and C_{20} and were identified based on diagnostic ions, retention time and the molecular peak. We calculated the total lipid content from the quantity of the internal standard and the quantity of all fatty acids together, and divided it by live body mass (mg) to obtain the relative lipid content.

The dataset of metabolic rate, body mass (both square-root transformation) and lipid content were separately analysed using linear mixed models (LMM). The relative frequency of social care compared to all other behaviours was assessed

using a GLMM following binomial distribution with a logit-link function. We always firstly examined whether uninfected ants from parasitized and unparasitized colonies differed in the metabolic rate, body mass, lipid content and social care. Hence, each model (LMERs & GLMM) included the colony parasitism (parasitized/ unparasitized) in interaction with ant category (infected/ nurse/ forager/ queen) as fixed predictors and colony identity as random factor. We applied a backward stepwise selection procedure for model selection ($\alpha = 0.05$). Neither metabolic rate, body mass, lipid content nor social care were impacted by the colonies' parasitism status (see results). We thus combined data for parasitized and unparasitized colonies, and additionally analysed whether and how the metabolic rate (LMER), body mass (LMER), lipid content (LMER) and social care (GLMM) differed between infected workers, nurses, foragers and queens. The ant category served as a single explanatory variable, and colony identity was included as random factor. Models were run in R using package lme4 (command lmer); (R Core Team 2012).

The interactive effect of Transferrin knockdown and paraquat-induced oxidative stress on worker survival

We explored the proposed antioxidant role of transferrin in workers of *T. nylanderi* using RNAi-mediated downregulation of *Transferrin* followed by a paraquat-induced oxidative stress response. Paraquat is a pesticide (N, N'-dimethyl-4,4'-bipyridinium) commonly used in experiments to induce *in-vivo* oxidative stress in plants, mammals and insects (Seehuus et al. 2006; Chen et al. 2010; Hosamani & Muralidhara 2013; Martin et al. 2018). Paraquat interferes with the redox cycling reactions inside cells, catalysing the formation of reactive oxygen species (ROS), which cause severe damage to macromolecules including lipids, proteins and DNA and enhance aging (Rzezniczak et al. 2011).

We delivered both treatments (dsiRNA and paraquat) via feeding. Custom dicer-substrate short interfering RNAs (dsiRNA) were synthesized by IDT (Integrated DNA Technologies, Inc.) and designed using the IDT's custom DsiRNA design tool (see supplementary material, **Table S4**). We collected 40 queen-right parasitized colonies with at least four infected workers between January and May 2017 (range: 4-33); (colony size of 73 ± 39: mean ± s.d.). Note that we could not control for worker age in the following experiments. Colonies were moved for one week to a temperature of 25°C without providing food to promote the uptake of dsiRNA by increasing ants' hunger level. Before starting the RNAi treatment, we wire-marked four infected workers, four nurses and four foragers in each colony (total of 12 workers per colony, $n = 480$). Half of the colonies were treated with dsiRNA for *Transferrin* (based on (Feldmeyer et al. 2016, Chapter 3)), while control colonies received dsiRNA similar in length and nucleotide composition but with no homologous region in the available *T. nylanderi* transcriptome. After one week of food deprivation, we offered each colony on a daily basis for 22 days, 15µl of sucrose solution including 0.1µg/µl per dsiRNA fragment (two fragments in total for *Transferrin*).

On day 14, each colony was evenly split (including the twelve marked workers), which resulted in 40 sub-colonies of the control treatment and 39 sub-colonies of the knockdown treatment. One colony was excluded as the majority of workers including marked ones had disappeared. The queen was removed from each colony to eliminate possible effects of queen presence on gene expression patterns of workers. Complete queens were homogenized in 100µl Trizol (Thermo Fisher Scientifics) and frozen at –80°C. On day 15, parallel to the dsiRNA delivery, we started applying onto the head of one of the marked individuals per ant category in each colony fragment either a droplet of rapeseed oil (control) or a single 0.5mM paraquat-oil droplet (treatment), using a stainless insect pin (bioform; size 1, 0.4 x 38 mm). The applied oil droplet induced self-grooming by which workers ingested paraquat (PESTANAL®, Sigma Aldrich). Survival differences between workers may depend on the absorbed amount of oil. We thus evaluated in pilot experiments whether workers absorb the same amount of oil using the presented method (Kruskal-Wallis-Test: $n = 21$, Δ d.f. = 20, $p = 0.46$). Treated workers were isolated for 30 mins before returning them to their colony fragment. Survival of individuals was observed on a daily basis for a total of eight days (i.e. from day 15 until day 23). Following the last scan, we sampled ants for qPCR analyses to confirm the downregulation of *Transferrin* (methods and results are given in the supplementary material). The delivery of dsiRNA through food has been successfully used to downregulate the gene *Vitellogenin-like A* by over 70% in the fat body in a related North American *Temnothorax* species (Kohlmeier et al. 2018a). Here, we were unable to show a downregulation of *Transferrin* (e.g. **Figure S2a**), but can demonstrate a clear effect of our treatment on worker survival (see below).

Differences in survival following dsiRNA and paraquat delivery were analysed in Cox proportional hazards regression models run in R (R Core Team 2012). We split the dataset into oil-treated and paraquat-oil-treated ants and analysed them separately. This resulted in two models, in each of which the RNAi treatment (knockdown/control) in interaction with the ant category (infected / nurse/ forager) were the explanatory variables and colony identity was a random factor.

Results

Long-term worker and queen survival

The comparison between parasitized and unparasitized colonies revealed that the presence of infected workers reduced survival of their uninfected nestmates (Cox model, colony parasitism: $\chi^2_2 = 8.42$, $p = 0.004$). Both uninfected nurses ($z = -1.66$, $p = 0.01$) and foragers ($z = -3.22$, $p = 0.001$) in parasitized colonies revealed a shorter lifespan than workers of these behavioural castes from unparasitized colonies (**Figure 17**). However, queen survival did not differ between parasitized and unparasitized colonies ($z = -0.71$, $p = 0.48$). Foragers from unparasitized colonies survived to the same extent as nurses ($z = 0.15$, $p = 0.88$), whereas in parasitized colonies nurses survived longer than foragers ($z = 2.04$, $p = 0.04$). Infected workers

and queens from parasitized colonies did not differ in survival (z = 0.07, p = 0.95) with both groups surviving considerably longer than nurses and foragers (Cox summary, nurse: z = 12.86, p < 0.0001; z = 9.14, p < 0.0001; forager: z = 14.18, p < 0.0001; z = 9.14, p < 0.0001; Cox model, ant category: χ^2_3 = 320.61, p < 0.0001); (**Figure 17**).

None of the 295 marked uninfected workers in parasitized colonies survived until the end of the observation period, whereas 52 of 103 (~ 51%) infected workers and 16 of 30 (~ 53%) queens were still alive after three years. In unparasitized colonies, none of the 140 nurses, only 3 of 140 foragers (~ 2%) and 17 of 28 (~ 61%) queens were alive after three years. Across parasitized and unparasitized colonies, uninfected nurses survived on average for 297 ± 217 days, uninfected foragers for 255 ± 218 days, infected workers for 825 ± 304 days and queens for 854 ± 314 days (mean ± s.d.). Overall, 12 of 58 (25.86%) colonies died within the three year observation period, but parasitized colonies were not more likely to die than unparasitized colonies (n_{para} = 8, n_{unpara} = 4, chi-square-test: χ^2 = 1.35, p = 0.25).

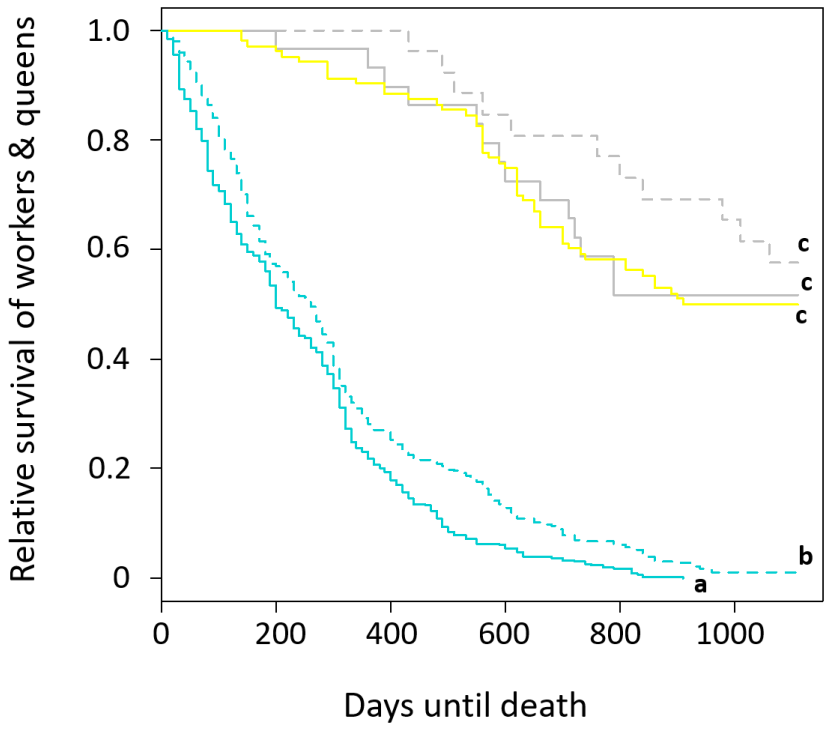

Figure 17. Survival of queens (grey), infected (yellow) and uninfected workers (cyan) of *T. nylanderi* over a duration of 1110 days. Ants from parasitized colonies are depicted with a solid line; ants from unparasitized colonies are represented by dashed lines. Different letters indicate significant differences between categories.

Metabolic rate, body mass and lipid content

Infected workers and nurses had similar metabolic rates (z = 1.16, p = 0.25), while queens had a lower metabolic rate (all $p < 0.0001$) and foragers a higher one (all $p < 0.03$; LMER, ant category: $\chi^2_3 = 96.62$, $p < 0.0001$; **Figure 18a**). Infected workers and nurses also had a similar body mass (z = 1.44, p = 0.16) and a similar relative lipid content (z = 1.65, p = 0.10); (**Figure 18b & c**). Queens had the highest body mass, while foragers had the lowest one (**Figure 18b**). However, both had similar relative amounts of lipids (z = 1.19, p = 0.24), but much less than infected workers (queen: z = - 3.21, p = 0.002; forager: z = - 2.10, p = 0.04) and nurses (z = - 5.98, $p < 0.0001$; z = - 4.52, $p < 0.0001$; **Figure 18c**), (LMER, ant category: $\chi^2_3 = 41.61$, $p < 0.0001$). Generally, ants from parasitized and unparasitized colonies did not differ in their metabolic rate, body mass and lipid content (LMER $_{\text{metabolic rate}}$, colony parasitism: $\chi^2_1 = 0.02$, p = 0.89, colony parasitism x ant category: $\chi^2_1 = 0.58$, p = 0.75; LMER $_{\text{lipid content}}$, colony parasitism: $\chi^2_2 = 0.14$, p = 0.15; colony parasitism x ant category: $\chi^2_2 = 1.25$, p = 0.54).

Social care in T. nylanderi

Social care provided towards infected workers exceeded that provided to both other worker castes and the queen (GLMM, ant category: $\chi^2_3 = 118.83$, $p < 0.0001$; **Figure 18d**). Foragers received less care than nurses (z = - 4.19, $p < 0.0001$) and nurses received less than queens (z = - 3.88, $p < 0.0001$). Despite the higher investment into infected workers, the care provided towards queen and other workers did not differ parasitized and unparasitized colonies (GLMM, colony parasitism: $\chi^2_2 = 0.03$, p = 0.87; colony parasitism x ant category: $\chi^2_2 = 2.85$, p = 0.24).

The interactive effect of oxidative stress and Transferrin knockdown on worker survival

Tapeworm-infected workers are known to overexpress *Transferrin* in the brain (Feldmeyer et al. 2016, Chapter 3). Our quantative real-time PCR analyses revealed that infected workers also upregulate *Transferrin* in the fat body (**Figure S2b**). In the whole body, the expression of *Transferrin* in infected workers matches that of queens (**Figure S2c**). Although infected workers usually lived longer than nurses and foragers, this difference was eliminated when the workers were exposed to oxidative stress simultaneously with a *Transferrin* knockdown.

Without oxidative stress, infected workers and nurses exhibited similar survival (z = 1.36, p = 0.18) and both worker types survived better than foragers (infected workers: z = 3.97, $p < 0.0001$; nurses: z = 5.22, $p < 0.0001$; Figure 19). The RNAi-treatment alone (i.e. without paraquat) had no effect on worker survival (Cox model: $\chi^2_1 = 1.05$, p = 0.31). As expected, oxidative stress induced by paraquat led to a high

mortality rate (**Figure 19a-c**). Both infected workers and nurses withstood this stress better than foragers (infected: z = 3.95, p < 0.0001; nurses: z = 3.20, p = 0.001), with no further difference between the two types in survival (z = 0.86, p = 0.39). Treating ants with *Transferrin*-dsiRNA and paraquat did not further reduce the survival of nurses (z = 1.40, p = 0.16; **Figure 19b**) or foragers (z = 0.80, p = 0.43; **Figure 19c**). In contrast, infected workers, which overexpress *Transferrin*, receiving both *Transferrin*-dsiRNA and paraquat died at higher rates than infected workers receiving paraquat alone (i.e. control-dsiRNA: z = 2.65, p = 0.008; Cox model, RNAi-treatment: χ^2_1 = 5.42, p = 0.02; RNAi-treatment x ant category: χ^2_2 = 6.3, p = 0.04; **Figure 19a**). Thus, during the combination of *Transferrin*-dsiRNA and oxidative stress treatment, infected workers exhibited mortality rates similar to that of nurses (z = 0.46, p = 0.64) and foragers (z= 0.59, p = 0.55).

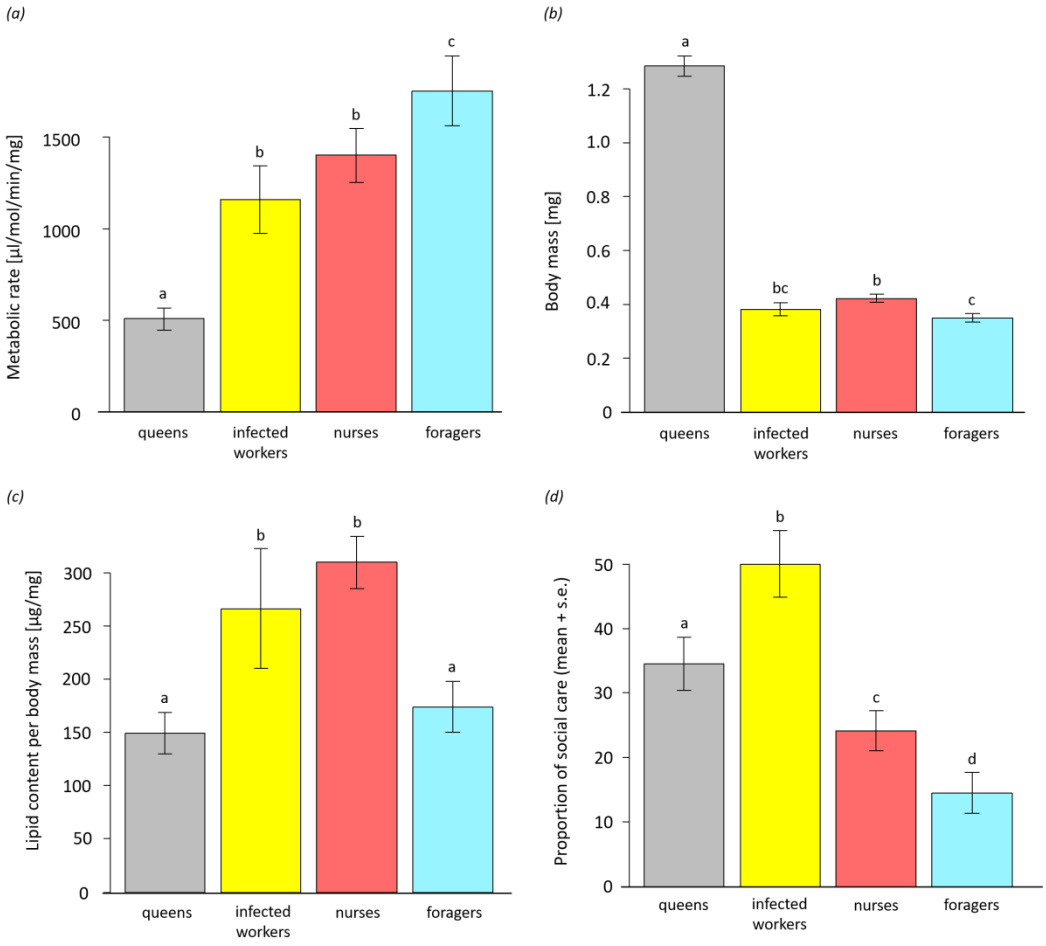

Figure 18. Differences in (***a***) metabolic rate, (***b***) body mass, (***c***) lipid content and (***d***) received social care between queens (grey), infected workers (yellow), nurses (red) and foragers (blue) from *T. nylanderi* colonies. Vertical lines represent standard error. Different letters indicate significant differences between categories.

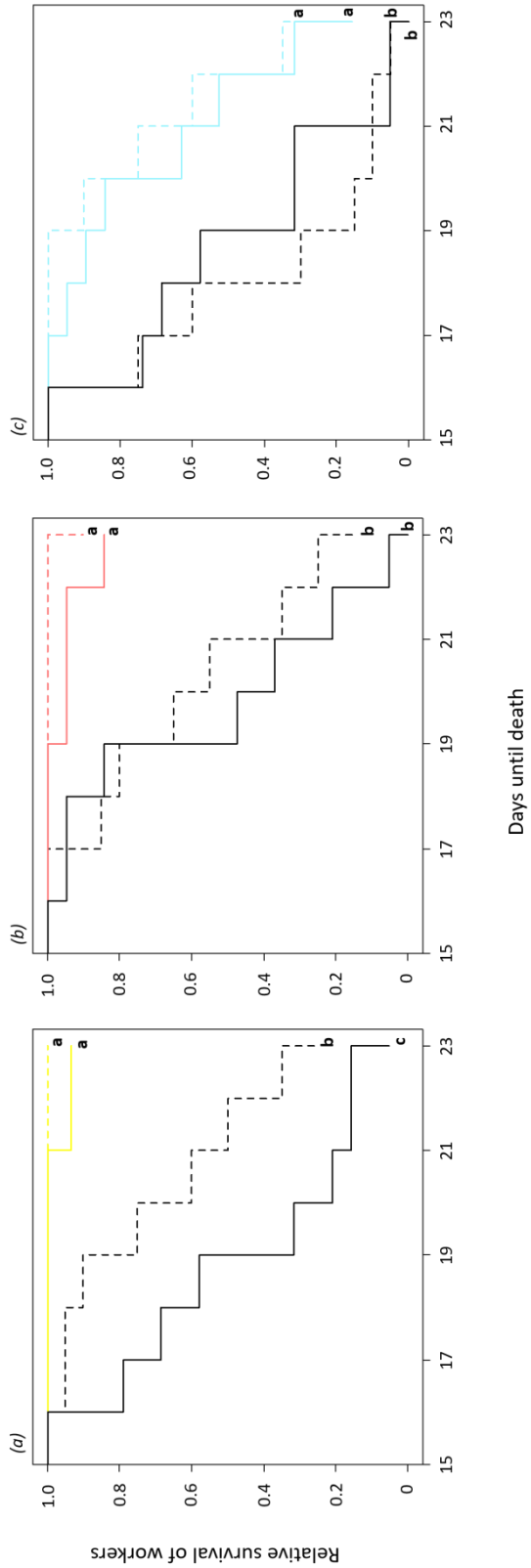

Figure 19. Survival during days 15 to 23 of infected workers (***a***), nurses (***b***) and foragers (***c***) in relation to the RNAi treatment (*Transferrin* knockdown – solid line, control – dashed line) and paraquat treatment (paraquat – black, oil – yellow; red or blue).

Discussion

Parasites induce numerous behavioural, physiological and life-history changes in their hosts, often manipulating the host in their own interest. We focused here on the trophically-transmitted tapeworm *A. brevis* and its intermediate host ant *T. nylanderi*. We show that infected workers live much longer than their uninfected nestmates. Indeed, at least during our three-year observation period, the survival of infected workers was similar to that of *T. nylanderi* queens, which can live up to two decades. However, uninfected workers in parasitized colonies demonstrated a shorter lifespan than workers in unparasitized colonies, reflecting a cost of parasitism inflicted on the colony as a whole.

The most striking finding is that of the extraordinary lifespan extension of the host ant induced by the tapeworm *A. brevis*. The observed survival differences are extreme, with more than half of all infected workers being still alive after more than 1000 days, while almost all uninfected workers had died. This more than three-fold elongation in lifespan exceeds that reported in other systems by far, such as the 40% increase in lifespan in mice infected with roundworms or flour beetles infected with tapeworms (Weatherly 1970; Hurd et al. 2001). The most apparent reason why infected workers can live so long may relate to the care provided by their nestmates. Indeed, infected workers did not only received more care than the average worker, but also significantly more than the queen, which is otherwise the most pampered ant in the colony. We initially predicted that tapeworm infection might lead to a reduction in lipid content and body mass and an increase in metabolism, which is a common, which is a common response in many animals fighting parasitic infections. However, infected workers exhibit similar metabolic rate, body mass and lipid content to those of nurses, which represent the youngest members of the colony. Furthermore, the cuticular hydrocarbon profile of infected workers resembles that of nurses more than that of older foragers (Beros et al. 2017, Chapter 2). Finally, their preferred location of infected workers in the nest resembled that of young nurses, which is close to the queen on the brood pile (Scharf et al. 2012b). Our data hence indicate that infected workers can maintain the physiological status of young workers and successfully counteract ageing over several years. Our findings moreover suggest that the extreme differences in survival cannot be explained by an aberrant metabolic rate or the lipid content alone. First, while infected workers survived longer than nurses, the two worker types displayed similar metabolic rate, body mass and lipid content. Second, infected workers survived as long as their mother queen, but possessed more lipids, a higher metabolic rate and a lower body weight. However, it is possible that by upregulating anti-ageing pathways, normally used by queens to fight ageing, infected workers can remain physiologically young. Although both queens and infected workers receive lots of social care, it is unclear how infected workers are able to solict so much support from their nestmates. It is possible that chemical signals emitted by infected workers elicit care. Whether and how the parasite is involved in the chemical or behavioural phenotype of its intermediate host will be the focus of future studies.

In many social insect species, workers engage in distinct tasks according to their age. The typical division of labour comprises young workers staying inside the nest and tending the brood, while old workers take over the risky tasks, such as foraging. However, other factors besides age, such as experience, stress and nutritional status influence the likelihood of foraging (e.g. Toth et al. 2005; Ravary et al. 2007; Smith et al. 2011; Franklin et al. 2012). Workers of *Temnothorax albipennis*, for instance, decide whether to forage based on their fat reserves (Blanchard et al. 2000; Robinson et al. 2012). Indeed, age and nutritional status are often linked. A recent experimental transcriptome study on the congeneric ant, *T. longispinosus*, disentangled age from behavioural task and demonstrates that nursing behaviour is linked to fat metabolism, while foragers upregulate genes involved in carbohydrate metabolism (Kohlmeier et al. 2018b). Although infected workers are closer in age to foragers than to nurses, they resembled nurses more closely concerning their physiological state. Unlike the lean, highly active foragers with their high metabolic rate that take on outside-tasks, infected workers are corpulent, possess a slow metabolism and remain inside the nest (Scharf et al. 2012b; Beros et al. 2019, Chapter 4).

Insect transferrin is mainly produced in the fat body and secreted into the haemolymphe to bind and transport iron to various target tissues (e.g. Lee et al. 2006b, Kim et al. 2008). *Transferrin* was found to be upregulated in the brain of *Camponotus* ant workers infected with the manipulative fungal parasite *Ophiocordyceps* (de Bekker et al. 2015), as well as in the brain of our tapeworm-infected *T. nylanderi* workers (Feldmeyer et al. 2016, Chapter 3). We show here that the expression of *Transferrin* is also higher in the fat body of infected workers compared to nurses. Moreoever, *Transferrin* expression in the whole body of infected workers matches that of the long-lived queens. We hypothesized that *Transferrin* may be involved in the protection against reactive oxygen species, as it has been shown to function as an anti-oxidant in other insects such as the beetle *Apriona germari* (Lee et al. 2006b). Indeed, the muscle atrophy and advanced age of infected workers suggest that they are more prone to the accumulation of oxidative amage than uninfected workers and should therefore activate responses to oxidative stress pathways (Feldmeyer et al. 2016, Chapter 3). Our dsiRNA experiment provides some evidence for the hypothesized role of *Transferrin* in parasite infection, as only the mortality of infected workers increased under oxidative stress in the *Transferrin*-knockdown treatment, whereas no such increase in mortality was observed in uninfected nurses and foragers. Because these uninfected workers express *Transferrin* only at very low levels, a further downregulation via RNAi would have been difficult to detect. Although application of RNAi produced a statistically significant alteration in the phenotype in infected workers in the expected direction, we failed to confirm a downregulation of this gene compared to control ants by RT-qPCR. Other RNAi studies have also revealed clear-cut phenotypic responses, but failed to confirm the downregulation (e.g. Cameron et al. 2013; Weiner et al. 2017). In our opinion, it is probable that our RNAi treatment was

successful, but downregulation could not be confirmed due to some of the following issues. For instance, ants treated with dsiRNA and paraquat or oil could not serve as qPCR samples. Instead, we had to use their nestmates from the same dsiRNA-treatment, where downregulation may have differed from that in the individuals treated with paraquat or oil (see supplementary material). Thus, we may have failed to detect a significant downregulation due to the sampling method. Moreover, our sample size for qPCR analyses may have been too low. Finally, we may have tested gene expression at an incorrect point in time: e.g., if if *Transferrin* downregulation had physiological consequences only early on in the experiment but not later on, even if the consequences remained. This is especially plausible given that workers may have avoided food or social interactions, especially after being challenged with paraquat. Infected workers do not feed by themselves and depend on the food provided by their nestmates. It is conceivable that uninfected workers might have stopped feeding them after day 15 and that infected workers therefore did not further receive dsiRNA to uphold the downregulation.

This study has not only revealed that infected workers exhibit a long-term survival that matches that of queens, but also that their nestmates, foragers and nurses alike, pay a high price of increased mortality. This cost of parasitism was apparent, despite the fact that the ant colonies were well maintained in our laboratory and regularly received ample food and water regularly. Possibly, the intense care for infected workers increases the workload of their nestmates, so that these workers become stressed. However, our physiological data did not point to increased physiological stress, as uninfected workers in parasitized colonies exhibited the same metabolic rate, body mass and lipid content as those from unparasitized colonies. Moreover, the survival of queens from parasitized colonies was unaffected by colony infection and workers also did not neglect the queen, although they provided much more intensive care for their infected nestmates. Field observations demonstrate that tapeworm parasitism does not negatively impact a colony's reproductive output, and naturally parasitized colonies are as often queenright as unparasitized colonies (Scharf et al. 2012b; Beros et al. 2019, Chapter 4). It might be in the parasite's interest that the queen is well cared for and that parasitized colonies can survive for many years, because only then might the parasite's survival and transmission be secured. Accordingly, we did not find any difference in colony survival in our three-year study.

In conclusion, our study demonstrates that a tapeworm drastically increases the survival of its social host. The physiology of old, infected workers is revealed as more similar to that of young nurses than to foragers or queens. Social attention and colony resources are directed towards infected workers. While this mitigates infection costs and may promote a long life, uninfected nestmates pay with a shorter lifespan. The next stage of research should be to study the importance of social interactions for the extended lifespan. Because nestmate care provided towards infected workers may be essential in prolonging their lifespan, social isolation should therefore be detrimental to their survival. If this explanation holds true, it

indicates that manipulative parasites can take advantage of their hosts' social system to prolong the life of their intermediate host in order to increase the likelihood of between-host transmission.

Author contributions

SB, FM and SF designed the experiments. SB, AL and MN performed the experiments. SB carried out the statistical and qPCR analyses. SB, IS and SF worte the paper. All authors gave final approval for publication.

Acknowledgements

[removed for privacy purposes]

Supplementary Material

Quantitative real-time PCR (qPCR)

We used marked untreated workers from each colony fragment for qPCR. None of these ants died during the entire experimental phase, implying that these workers might have not interacted with paraquat- and oil-treated ants. Wire-labels were carefully cut off, and ants were dissected into head, thorax (including legs) and abdomen. Each body part was separately homogenized in 50µl TRIzol, but we only analysed abdomen samples as transferrin is produced mainly in the fat body and secreted into the haemolymph (Lee et al. 2006, Kim et al. 2008). Real-time quantitative PCR was performed for 16 abdomen samples from tapeworm-infected workers and 16 abdomen samples from uninfected nurses (each $n_{\text{knockdown}} = 8$, $n_{\text{nonsense}} = 8$). In addition, we prepared 24 whole-body samples of tapeworm-infected workers ($n_{\text{knockdown}} = 12$, $n_{\text{nonsense}} = 12$) and 32 whole-body samples of queens receiving dsiRNA until day 14^{th} ($n_{\text{knockdown}} = 16$, $n_{\text{nonsense}} = 16$)

RNA was extracted using the Qiagen Mini Kit (ID: 74106), followed by cDNA synthesis using Qiagen's QuantiTect Reverse Transcriptase Kit (ID: 205313). Along with *Transferrin*, we analysed the house-keeping gene *GADPH* (glyceraldehyde 3-phosphate dehydrogenase) using SYBR Green (Biozym® Blue S´Green qPCR Kit) and the MIC Real Time PCR Cycler from BMS (Bio Molecular Systems©). For tapeworm-infected workers, we calculated ΔΔCT values and compared the relative expression of the targeted gene between knockdown- and control-treated individuals using a Wilcoxon test (**Figure S2a**). Furthermore, we compared the relative expression of *transferrin* between tapeworm-infected workers and nurses (**Figure S2b**) and the tapeworm-infected workers and queens (**Figure S2c**).

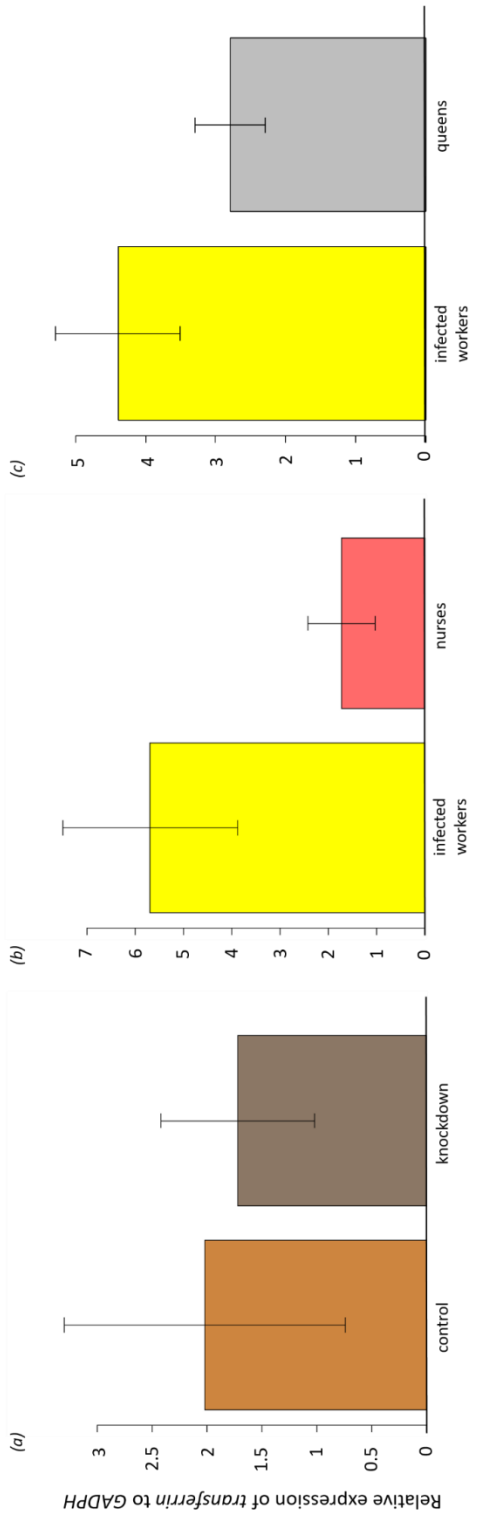

Figure S2. (***a***) Comparison of *Transferrin* expression relative to the housekeeping gene *GADPH* in infected workers, either treated with control-dsiRNA (light brown) or dsiRNA for *Transferrin* downregulation (dark brown), (W = 36, p = 0.72). (***b***) Comparison of *Transferrin* expression between control-treated infected workers and control-treated uninfected nurses

(*W* = 74, *p* = 0.047) and (***c***) control-treated infected workers and control-treated queens (*W* = 123, *p* = 0.22).

Table S4. Information on customized dsi-RNA sequences.

Fragment	**Direction**	**Sequence**
Transferrin _Frag 1	Sense	CUCCCUCGAAGAAUUGAAACAUGGC
	Antisense	GCCAUGUUUCAAUUCUUCGAGGGAGUU
Transferrin _Frag 2	Sense	CGAUAUGACUGCAUCGAGAGAGUCG
	Antisense	CGACUCUCUCGAUGCAGUCAUAUCGGU
Nonsense	Sense	UAGUACACGUCAUUGGAAUUGCAGC
	Antisense	GCUGCAAUUCCAAUGACGUGUACUAUU

General Discussion

Sara Beros

In this dissertation, I examined different aspects associated with parasitism by manipulative parasites in social hosts focusing on the host-parasite system of the trophically-transmitted tapeworm *Anomotaenia brevis* and its intermediate host, the ant *Temnothorax nylanderi*. One aim was to investigate the tapeworm-induced phenotypic alterations and the underlying genetic patterns in host ants. Additionally, I studied the consequences of tapeworm parasitism on the ant's colony level and took a closer look at potential costs, the behaviour and physiology of uninfected nestmates, and the colonies' chemical signature. This thesis reveals that tapeworm-infected ants are deeply modified social insect workers and demonstrates that the presence of workers infected with a 'manipulative' parasite affects their nestmates' behaviour and lifespan. The following discussion is divided into two major parts. The first part deals with the multiple phenotypic and genetic alterations and their implication for parasite transmission, and ends with an excursion to future research highlighting relevant approaches to study the *extended phenotype*. The second part focuses on the colony-level consequences of tapeworm parasitism and their implication for colony fitness and survival, closing with remarks to future projects.

Part I

Host manipulation is ubiquitous, extremely diverse and many times multidimensional (Cézilly & Perrot-Minnot 2005; Thomas et al. 2010). Single parasites can alter several phenotypic traits in their hosts, many times simultaneously or in succession (Cézilly & Perrot-Minnot 2005; Thomas et al. 2010, Cézilly et al. 2013). The tapeworm parasite *Anomotaenia brevis* fits this description well. It induces simultaneous alterations in behaviour, morphology, physiology and life-history of its intermediate host, the ant *Temnothorax nylanderi.* Adult ants infected with *A. brevis* are smaller and less pigmented, and express an aberrant hydrocarbon profile, which occasionally elicits aggression in other *T. nylanderi* workers (Trabalon et al. 2000; Scharf et al. 2012b; Chapter 1). The endoparasitic infection reduces the behavioural repertoire of ant workers. Tapeworm-infected workers are mostly inactive inside the nest, forage rarely and do not flee upon destruction of the nest (Scharf et al. 2012b, Chapter 2 & 4). Finally yet importantly, they outlive uninfected workers and thereby exhibit an extraordinary long lifespan for social insect workers (Chapter 5).

The best strategy for trophically-transmitted parasites to ensure transmission is to predispose the current host to predation by the next host, for instance by reducing or eliminating the current host's anti-predatory behaviours. Such a remarkable alteration is seen in *Toxoplasma*-infected rodents, which are attracted specifically towards their natural feline predators (Vyas et al. 2007; Lamberton et al. 2008). Alterations like these are highly specific and may clearly increase the predation of the intermediate host by the correct next host. Yet, many parasite-induced behavioural alterations are actually more subtle and less specific (Poulin 1994; Lafferty & Shaw 2013). Non-specific alterations in behavior could be costly for the

parasite, if they lead to predation by non-host predators. This is observed in trematode-infected cockles, which are more exposed on the sediment surface and are thus more vulnerable to predation by oystercatchers, the parasite's targeted host, but also to fish and whelks, both non-hosts unsuitable for parasite reproduction (Mouritsen & Poulin 2003a & b). Tapeworms and other helminths commonly alter the activity of their intermediate hosts (Hurd & Fogo 1991; Poulin 1994; Lafferty & Shaw 2013), and the same is true for the tapeworm, *A. brevis* (Scharf et al. 2012b; Chapter 4). Decreased activity combined with a reduced escape response of tapeworm-infected workers might secure predation by woodpeckers, the parasite's definitive hosts, and thereby facilitate parasite transmission (Chapter 1). Yet, altered activity levels are unspecific and tapeworm-infected ants could be exposed to predation by non-host predators. Nevertheless, non-specific behavioural alterations can evolve, if they generally increase the overall predation when initial predation is low, and therefore increase the likelihood of successful parasite transmission (Poulin et al. 2005; Seppälä & Jokela 2008; Seppälä et al. 2008). Accordingly, more specific host manipulation should evolve, if the initial predation risk is already very high (Seppälä & Jokela 2008). That said, my investigations reveal that infection increases the host's lifespan and tapeworm-infected workers might even live for several years (Chapter 1 & 5). In fact, *Temnothorax* ants can survive several months up to years in the field, which suggests that they have low predation risks (e.g. Keller 1998; Pamminger et al. 2012). Moreover, *T. nylanderi* ants frequently relocate their nest in the field, sometimes moving from a soft, unstable acorn to a rock-hard branch on the forest floor, which might impede predation (Foitzik & Heinze 1998; S. Foitzik pers. communication). Accordingly, the extended lifespan would be in the interest of the parasite in order to prolong the period of possible transmission, because predation by woodpeckers might be actually rare.

Previous work and work presented in this thesis show that tapeworm-infected workers rarely leave the nest (Scharf et al. 2012b, Chapter 1, 4 & 5). In contrast, health-comprised workers of *Temnothorax unifasciatus* desert their colony to die in isolation (Heinze & Walter 2010). This kind of behaviour, which is observed in other social insects as well, is often interpreted as an altruistic act, because the infected worker sacrifices her life to circumvent the risk of infecting her nestmates (e.g. Rueppell et al. 2010; Bos et al. 2012; Leclerc & Detrain 2017). Yet, we also find that some fungal pathogens, trematodes and nematodes force their hosts to withdraw from the nest to increase the parasites' chance to complete their life cycle (Carney 1969; Yanoviak et al. 2008; Loreto et al. 2014). Thus, if manipulative parasites are able to mediate social isolation in their interest, they may also induce avoidance to leave the nest. In my study system, self-removal would benefit the colony, but jeopardize the tapeworm's chance for transmission. Reduced foraging possibly benefits the parasite in a way that tapeworm-infected workers avoid situations which might put the parasite's life at risk. Foraging tapeworm-infected workers might be easy prey for non-host predators due to their sluggish behaviour and the less sclerotized cuticle. The latter might protect less well against desiccation and increases the chance of injuries and infestation by other parasites and pathogens, for

instance by entomopathogenic fungi, bacteria or viruses (de Bekker et al. 2018). It is likely that woodpeckers successfully prey on ants in their nest site rather than hunting tiny *Temnothorax* ants wandering between the thick leaf-litter on the forest floor. Probably, woodpeckers are primarily not interested in adult, chitin-rich ants, but in their brood or in larvae of other insects nesting in deadwood. If this is true, it could certainly explain why tapeworm-infected workers do not move away from the brood.

While some alterations seem to be adaptive for *A. brevis*, there are a number of traits that do not obviously contribute to the tapeworm's transmission, for instance the altered chemical profile and the increased reproductive potential of infected workers (Trabalon et al. 2000; Chapter 1; 2 & 4). In fact, the hydrocarbon composition of tapeworm-infected workers elicits aggression and this might lead to fatal injuries (Chapter 1). The increased reproductive potential, on the other hand, boosts exclusively the direct fitness of infected hosts (Chapter 4). The most apparent alteration in *A. brevis*-infected ants is the altered cuticle pigmentation (Plateaux 1972; Moore 1995; Trabalon et al. 2000; Scharf et al. 2012b). Unlike their brownish conspecifics, tapeworm-infected workers of *T. nylanderi* are completely yellow (Trabalon et al. 2000). Also in other *Temnothorax* species, such as *T. rugatulus, T. affinis* and *T. longispinosus*, infections with different tapeworm species lead to a lighter, less sclerotized cuticle (Heinze et al. 1998, S. Foitzik pers. communication). In two *Myrmica* species, however, tapeworm infection darkens the cuticle (Muir 1954). Indeed, parasite-induced changes in body coloration and appearance are quite common and can be a very effective approach to attract the attention of predators, especially when targeted hosts such as birds and fish are visually oriented (e.g. Bakker et al. 1997; Yanoviak et al. 2008; Wesołowska & Wesołowska 2014). A very striking example is given in the giant turtle ant infected with a nematode. The parasite changes the ant's abdomen from black to deep red, so that it resembles a berry eaten by frugivorous birds (Yanoviak et al. 2008). Apart from that, coloration does not necessarily increase the vulnerability of hosts (Kaldonski et al. 2009) and vivid coloration can be also used by parasites to scare off predators (Fenton et al. 2011). Finally, colour changes can be part of the host's immune response as for example found in coral polyps infected with the trematode *Podocotyloides stenometre* (Palmer et al. 2009). Infected polyps turn pink and attract butterfly fish, which preferentially feed on the coral's tissue (Abey 2002; 2003). Yet, the pink pigmentation is caused by a red fluorescent protein produced by the host and helps the coral to eliminate damaged tissue by exposing it to predators (Abey 1992; Palmer et al. 2009). This example strongly implies that parasites are able to exploit their hosts' compensatory responses, which seems to be an effortless strategy for parasites (e.g. Lefèrve et al. 2008).

Whether the bright, yellow coloration of *A. brevis*-infected ants enhances their predation is not yet clear. More importantly in this context, if the colour change is truly favouring parasite transmission, *A. brevis* should be able to induce it under all circumstances. However, a proportion of tapeworm-infected workers resemble the

brownish, healthy looking phenotype, which indicates that the colour change is a by-product of infection (Scharf et al. 2012b). In insects, melanization and sclerotization are mechanistically linked with the immune system (e.g. Barnes & Siva-Jothy 2000, Cotter et al. 2004). Phenoloxidase (PO) is the key enzyme of the insect's innate immune system and involved in the synthesis of melanin, which is further produced to encapsulate parasites and pathogens (True 2003). Alive cysticercoids of *A. brevis* indicate that the tapeworm is able to circumvent encapsulation. One idea is that *A. brevis* interferes with its host immune system, possibly inhibiting the activity of PO, which could result in a lower degree of cuticular melanization and sclerotization.

The plentiful examples of host alterations, including the study system of this work, illustrate nicely that manipulation by parasites is a catchy concept and that infections with manipulative parasites altering multiple host traits result in a complex "infection syndrome" (Cézilly et al. 2010). Parasite-induced alterations are traditionally interpreted by three different hypotheses that are mutually exclusive: serving either the parasite, the host or being side-effects of infection with no adaptive value for both biotic partners. The differentiation between the three explanations has proved to be challenging (e.g. Poulin 1995; Thomas et al. 2005; Poulin 2010), not least because of the multidimensionality of host manipulation (Thomas et al. 2010; Cézilly & Perrot-Minnot 2010; Cézilly et al. 2013). Future research should shed more light on which alterations are truly adaptive for *A. brevis*, and therefore part of its *extended phenotype* (Thomas et al. 2010). On the other hand, it should be kept in mind that multiple traits might act synergistically to facilitate parasite transmission independent of their origin (Cézilly et al. 2013). Recently, it has been recognized that altered hosts are not illustrations of a total parasite takeover. Alterations should be rather viewed as co-evolved traits and can represent compromises between parasite and host (e.g. Lefèrve et al. 2008 and citations within).

Two key pieces of evidence are required to confirm that host alterations are truly in favour of the parasite. Firstly, a positive link between the altered phenotype of the host and the fitness of the parasite has to be provided by experimental evidence (e.g. Poulin & Maure 2015). Secondly, because natural selection acts on the *parasite's ability* to manipulate its host, not on the host's phenotypic alterations, the mechanism(s) utilized by parasites to manipulate their hosts need(s) to be uncovered (Cézilly et al. 2010). The latter is currently subject of intense research and has to follow approaches that can generate evidence confirming adaptive manipulation by the parasite on the one hand, and simultaneously ruling out alternative explanations on the other hand (Herbison et al. 2018). Investigating the molecular mechanisms behind host manipulation over the last two decades, has revealed that pathways utilized by parasites are extremely diverse, can be more or less sophisticated and depend strongly on the specific host-parasite interaction (e.g. Lefèrve et al. 2008; Adamo 2013; Lafferty & Shaw 2013; Hughes & Libersat 2018). Behavioural alterations can be induced by parasites through direct or indirect interaction with the host's central nervous system, and through energetic drain or

tissue damage (e.g. Adamo 2013; Lafferty & Shaw 2013; Hughes & Libersat 2018). As a matter of fact, a third biotic partner can be brought to the table. This is for instance the case in the ladybug, *Coleomegilla maculata,* which serves its parasitoid wasp, *Dinocampus coccinellae*, as a bodyguard, but is actually manipulated by a symbiont of the parasitoid, a RNA virus (Dheilly et al. 2015). Some parasites directly interfere with the host's central nervous system, as it is the key organ for coordinating behaviour (e.g. Rosenberg et al. 2006; Berenreiterová et al. 2011; Martín-Vega et al. 2018). However, the majority of parasites are not located in the host's brain, but nevertheless capable of spectacular behavioural alterations (e.g. Høeg 1995; Thomas et al. 2002; Geffre et al. 2017). This indicates that the site of infection does not limit a parasite's ability to alter host behaviour, rather the mechanisms behind the alterations are of importance (Poulin 1994; Lafferty & Shaw 2013).

Most helminth propagules, including *A. brevis*, reach their intermediate host via oral uptake and migrate to the body cavity, where they usually trigger a rapid encapsulation process mediated by the host's immune system (e.g. König & Schmid-Hempel 1995). The tapeworm *Schistocephalus solidus* is able to reduce the probability of recognition by its host's immune system by losing its outer layer and adjusting the carbohydrate composition of its new surface to its fish host while entering its body cavity (Hammerschmidt & Kurtz 2005). Besides, *S. solidus* induces a strong immune response and additionally activates genes involved in neural pathways and sensory perception when being ready for transmission to the definitive host (Scharsack et al. 2007; Hébert et al. 2017). Upregulated leucocytes do not help to eliminate the tapeworm, but their activation might interfere with the host's neuroendocrine system and thus mediate the behavioural alterations, which facilitate parasite transmission (Scharsack et al. 2007). In my study system, I find only little evidence that cysticercoids of *A. brevis* activate or supress the ant's immune system (Chapter 3). Apart from the upregulation of the gene *Transferrin*, which is proposed to be involved in the immune response of social insects (e.g. Kucharski & Maleszka 2003; Thompson et al. 2003; Valles & Pereira 2005), no other immunity-related genes are differently expressed in the presence of cysticercoids of *A. brevis* (Chapter 3). Consequently, *A. brevis* might be able to evade the ant's immune system in a similar way as *S. solidus*. However, ants eventually do not invest in a strong immune response as tapeworm larvae are probably too big to be destroyed. Finally, my results imply that infective cysticercoids of *A. brevis* do not stimulate the immunomodulatory pathway, and there is no evidence for a differently expression of certain neuromodulators (Chapter 3).

Parasites have been selected to successfully exploit their host's resources. Tapeworm-infected workers are smaller (Trabalon et al. 2000; Scharf et al. 2012b), but are not nutritionally or energetically challenged; meaning tapeworm-infected workers are corpulent, can quickly develop their ovaries and have similar metabolic rates as nurses (Chapter 4 & 5). Interestingly, also *Camponotus* ants infected with the lancet liver fluke, *Brachylecithum mosquensis*, are well nourished (Carney 1969).

These findings may suggest that ants experience more nutritionally challenges during their early larval development, while the parasitic eggs are growing and transforming into infective cysticercoids. This assumption is supported by findings in another tapeworm species. The previously mentioned tapeworm *S. solidus* exploits its vertebrate host's resources and activates genes related to parasite growth, cell division and regulatory processes mainly during its transition from a pre-infective to an infective plerocercoid stage (Hafer & Milinski 2015b; Hébert et al. 2017).

In *T. nylanderi* workers, infection is associated with the downregulation of muscle genes resulting in muscle atrophy (Chapter 3). Although this is only confirmed for the mandibular muscles, one may assume that muscles of other body parts (e.g. legs & thorax) are likewise affected. Tapeworm-infected workers may not be able to walk long distances or transport heavy food items, brood or other nestmates, because of their reduced and deformed muscles. Hence, muscle atrophy could explain the overall reduced activity in tapeworm-infected workers of *T. nylanderi* (Chapter 4). Behavioural alterations due to tissue damaging have also been reported in aquatic hosts of two different trematode species. The first trematode parasite, *Diplostomum spatheceum,* encysts in the eye of its fish host inducing a cataract (Seppäla et al. 2004). Consequently, the host is impaired in its vision and swims closer to the water surface, where it becomes more susceptible to predation by birds (Seppälä et al. 2005). The other trematode, *Curtuteria australis*, accumulates in the foot of its cockle host and damages the muscle tissue. Invested cockles are no longer able to bury themselves deep enough into the ground to hide effectively from predators such as oystercatchers (Thomas & Poulin 1998; Mouritsen 2002). Unlike trematodes that migrate within their host's body and are the direct cause of the damaged tissue, the tapeworm *A. brevis* resides exclusively in the ant's body cavity. It has been suggested that oxidative processes are involved in the degeneration of flight muscles in queens of the fire ant *Solenopsis* spp. (Davis et al. 1993). Since tapeworm-infected workers may reach an old age, it is very likely that they experience more oxidative stress than other workers (Chapter 5). Thus, one suggestion would be that oxidative stress is responsible for the reduced and damaged muscle tissue in old, tapeworm-infected workers. In line with this, I find several genes associated with the regulation of oxidative processes to be differently expressed (e.g. *Cytochrome C, Tropomyosin, PHGP* and *Transferrin*, but also see Chapter 3). Moreover, our investigations show that the gene *Transferrin* is somehow involved in the response towards oxidative stress and might limit deleterious effects caused by reactive oxygen species (ROS).

The most striking alteration in *T. nylanderi* is the increased lifespan (Chapter 1 & 4). Parasites can induce such alterations in the course of resource exploitation. Hosts usually have fewer resources available and can only devote these to one trait causing a trade-off in other traits, such as that reduction in fecundity increase longevity (Agnew et al. 2000). In a different insect-tapeworm system, survival of female beetles is primarily increased, because host fertility is reduced by the

tapeworm *Hymenolepis diminuta* (Hurd et al. 2001). However, this is not a possible explanation for my study system, because tapeworm-infected workers seem to have enough resources available and can easily allocate them into their ovary development (Chapter 4). By definition, trophically-transmitted parasites decrease their host's lifespan by facilitating predation. Nevertheless, recent investigations provide evidence that parasites can supress predation and hence, increase hosts' survival. This is necessary when parasites are still in their early development and predation would be detrimental for parasites' survival (e.g. Dianne et al. 2011; Weinreich et al. 2013). Again, tapeworms of *A. brevis* have already reached their infective stage, yet ants' survival is prolonged for several years, which suggests that a longer life expectancy may be necessary to increase the probability of transmission (see also earlier discussion). In all social insects, the reproductive queen is the long-lived unit, while the usual sterile workers live only several weeks or months (e.g. Negroni et al. 2016). The life expectancy of tapeworm-infected workers exceeds that of other, uninfected workers in this species (Chapter 5). While the infection with *A. brevis* increases the survival of hosts, it reduces the life expectancy of their nestmates (Chapter 1 & 5). These results strongly indicate that the maintenance of tapeworm-infected workers is energetically costly and social care might be a major factor that contributes to the extended survival.

A very intriguing aspect in our host species is the absence of age-related behaviours (e.g. foraging; Scharf et al. 2012b; Chapter 4). Many bees and ants exhibit age-related biases in task performance. Usually, younger social insect workers start their life with in-nest tasks and progress to perform riskier tasks such as foraging with increasing age (Robinson 1992). Moreover, some social insects tend to withdraw from their colony upon infection with parasites, which implies that the health status of an individual can determine certain tasks (Morón et al. 2008; Tofilski 2009; Heinze & Walter 2010). The opposite is observed in *T. nylanderi*: old, infected workers remain inside the nest and rather exhibit the behavioural and physiological phenotype of young nurses (Chapter 4 & 5). Interestingly, few studies demonstrate that parasites accelerate the behavioural maturation of social insects and this might be associated with an altered responsiveness to social and environmental cues (Lecocq et al. 2015; Natsopoulou et al. 2016; Leclerc & Detrain 2017). Applied to my study system, it would suggest that *A. brevis* is decelerating the age polyethism in its host ant. In accordance with that, we find three copies of the gene *Vitellogenin* to be differently expressed upon infection with *A. brevis* (Chapter 3). One of them, *Vitellogenin-like A* (formerly *Vitellogenin-6*), has recently been found to be involved in the responsiveness to ant brood cues (Kohlmeier et al. 2018a). In line with this, *Vitellogenin-like A* is uniquely upregulated in tapeworm-infected workers, which fits their behavioural profile, because tapeworm-infected workers are highly affine to brood (Scharf et al. 2012b; Chapter 4). Hence, *Vitellogenins* could be good candidate genes to study, if *A. brevis* is partially using this mechanism to induce certain behavioural alterations. Apparently, the tapeworm *Hymenolepis diminuta* actively releases a yet unidentified substance that changes the synthesis of vitellogenin (Webb & Hurd 1999; Warr et al. 2006).

Part I - Conclusion and outlook

A significant part of this PhD thesis has dealt with the altered phenotype and the underlying genetic mechanisms in tapeworm-infected *T. nylanderi* workers*.* I propose that the lethargic behaviour and the extended lifespan might be in the interest of the tapeworm to complete its life cycle. The phenotype may be explained by a complex interplay between the underlying expression of certain genes (e.g. *Transferrin* & *Vitellogenin-like A*), muscle atrophy and the high rates of social care (Chapter 3 & 5). As findings in this thesis mainly provide correlative and lack causative evidence, I am limited in the interpretation of adaptive effects. However, the findings equip future projects with essential knowledge and should help to postulate testable hypothesis to separate causes from consequences. Generally, future research has to focus more on the molecular level and on the genetic basis of the parasite, for instance by using transcriptomic and proteomics approaches. This is currently done in an ongoing study in this host-parasite system (Stoldt et al., in preparation). Preliminary results suggest that cysticercoids of *A. brevis* are transcriptionally very active and release numerous proteins into their host's haemolymph. The vast majority of haemocoelian proteins presumably originate from the tapeworm (**Figure 20**). Remarkably, tapeworm proteins circulate also in the haemolymph of uninfected nestmates indicating that these workers were once infected but managed to clear the infection or tapeworm-infected nestmates actually transfer parasite-derived proteins during fluid transfer (LeBoeuf et al. 2016). Next steps should be taken to determine the function of proteins and to elucidate their role in the molecular cross-talk between tapeworm and ant. Because *A. brevis* resides in its ant's body cavity, it is in a good position to release one or several molecules that directly communicate with host's physiology. Thus, particular effort should be made to identify the '*factor*' produced and released by *A. brevis*, which directly interacts with the ant's physiological system.

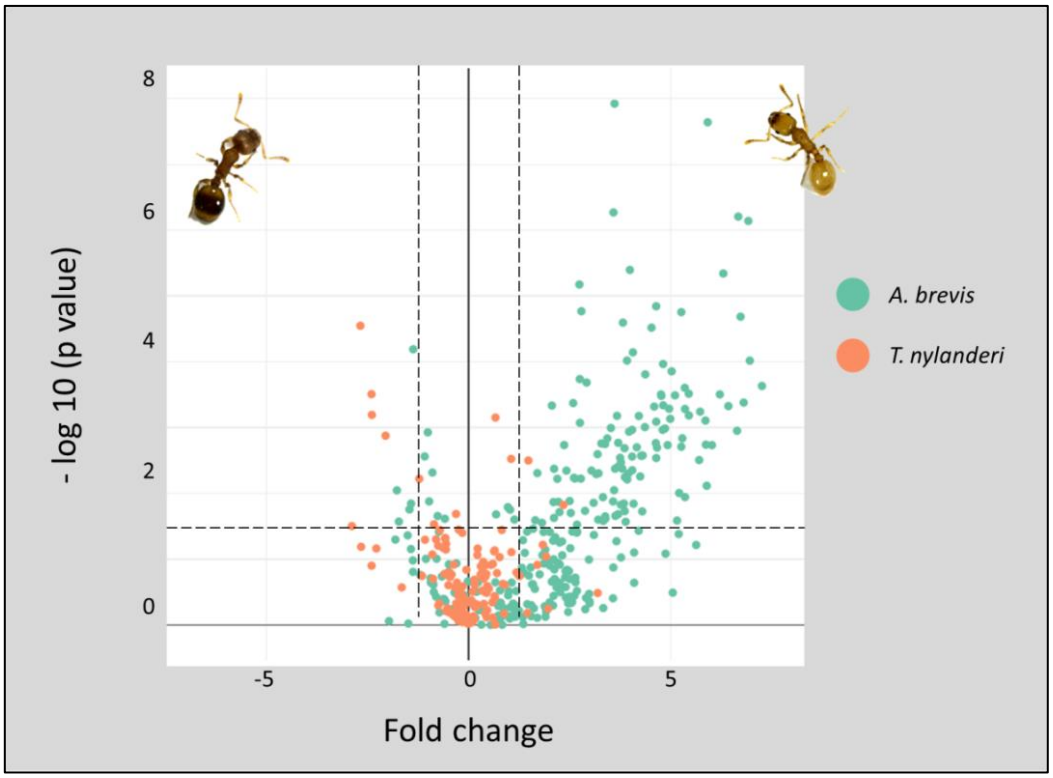

Figure 20. Volcano Plot illustrating different protein species in the haemolymph of tapeworm-infected workers (right) vs. their uninfected nestmates (left). Proteins derived from the tapeworm are shown in green. Proteins of the ant *T. nylanderi* are orange. The log fold change is represented on the x-axis. The y-axis shows the log 10 of the p value. A p value of 0.05 and a fold change of 2 are indicated by the dashed, black lines.

Part II

The social organisation of insect societies, notably in ants, is considered the major reason for their tremendous ecological success (Wilson 1978). At the same time, it makes social insects more prone to exploitation by parasites and pathogens (Schmid-Hempel 1998; Boomsma et al. 2005; Cremer et al. 2007). Workers of *T. nylanderi* ants are frequently infected with the trophically-transmitted tapeworm *A. brevis* and I find that these infected workers are readily accepted in ant colonies and indirectly reduce the aggression of nestmates (Chapter 1). However, their presence does not broaden the colonial chemical profile that might have explained the lack of rejection (Chapter 1 & 2). Tapeworm-infected workers appear selfish as they do not actively engage in important colony-related tasks (e.g. foraging, defence, sanitary behaviours), but request all-around care from their nestmates (Scharf et al. 2012b, Chapter 1 & 4). Instead of isolating tapeworm-infected workers from colony resources, they receive the best social care and their nestmates suffer from higher mortality (Chapter 1 & 5).

Infected animals of a social group represent multiple risks. For once, they may initiate an epidemic by transmitting pathogens to other nestmates. Additionally, they may divert a significant amount of their colonies' resources towards their own well-being (Cremer et al. 2007). The larvae of *A. brevis* are trapped inside the ant's body cavity and adult tapeworm-infected workers do not represent an urgent source of infection risk (see also Chapter 4). Tapeworm-infected workers, however, increase trophallaxis and I additionally demonstrate that they receive more social care than the sole queen, which is not only the highest-ranking, but also the best-protected individual in an insect society (Chapter 5). Although workers take better care of tapeworm-infected workers, they do not neglect the queen, which explains that her survival is not affected by parasitism (Chapter 5). However, workers of parasitized colonies apparently pay a price, as I can show that they die earlier and at higher rates (Chapter 1 & 5). While workers may be easier to replace, the queen is irreplaceable. Presumably, her survival may also be in the interest of the tapeworm, because only in the presence of the queen, the colony will persist and new workforce can be produced that will continue to take care of tapeworm-infected workers (Chapter 5).

My findings strongly indicate that tapeworm-infected workers drain their colony's resources and that their maintenance is associated with serious costs. Yet, surprisingly, parasitized colonies occur at high frequencies and are larger (Scharf et al. 2012b; Chapter 5). Eventually, social insects adjust life-history traits such as reproduction to mitigate the costs (e.g. Buechel & Schmid-Hempel 2017). One idea is that queens of parasitized colonies might start to produce more workers to compensate for the loss. Thus, tapeworm parasitism would indirectly increase the reproductive output of ant queens (e.g. Giehr et al. 2017). However, earlier research has documented that *per-capita* productivity, an important fitness correlate in social insects, is not increased or reduced by tapeworm parasitism under field conditions,

but parasitized *T. nylanderi* colonies shift their investment strategies (Scharf et al. 2012b). Naturally parasitized colonies produce more and heavier males, and also more inter-caste individuals (Scharf et al. 2012b). Inter-castes are morphologically intermediate between the queen and the worker caste (Okada et al. 2013). The causes for their existence are not yet fully understood, but one assumption is that they experience an abnormal development due to nutritional deprivation. The higher percentage of inter-castes would therefore imply that ants of parasitized colonies try to raise more sexuals, but obviously struggle with the higher energetic demand for the production of gynes and males.

What are the causes for the increased mortality? - Survival is generally influenced by intrinsic and extrinsic factors. Currently, my findings point to the possibility that the high social investment in tapeworm-infected workers could be energetically exhausting. Remarkably, I do not find any evidence that nestmates experience elevated physiological stress that could explain their higher death rates. The metabolic rate and fat reserves of workers and queens from parasitized colonies are comparable to conspecifics in unparasitized colonies (Chapter 5). Nurses and foragers of parasitized colonies are not impaired in their ovary development and do not signal possible physiological changes on their cuticle profile (Chapter 2 & 4). Lastly, none of the 56 uniquely expressed genes in uninfected workers from parasitized colonies hint at significant metabolic differences (Chapter 3). However, it needs to be mentioned that survival costs were discovered under laboratory conditions and food supplies were limited. Eventually ants were unable to buffer nutritional costs due the lack of unlimited food, which points out that ants possibly employ different behaviours in the field that help to compensate fitness losses.

Based on findings presented in this thesis, it is reasonable to assume that tapeworm parasitism should exert a strong selection to evolve effective defences. The first line of defence is commonly parasite avoidance, for instance by avoiding or rejecting food sources contaminated with parasites propagules (Boomsma et al. 2005; Cremer et al. 2007). In some populations, up to one-third of the *T. nylanderi*-colonies are parasitized and due to a low percentage of infected workers exhibiting the normal phenotype, intra-colonial parasitism rates might be much higher than anticipated (Scharf et al. 2012b). The high infection rates imply that ants are not unable to detect helminth propagules in bird droppings. Most ants get infected during their larval development (Gabrion et al. 1976; Trabalon et al. 2000). Adult, tapeworm-infected workers could remove themselves as an altruistic act, yet they remain inside the nest (Scharf et al. 2012b; Chapter 1 & 4). Because infection supposedly cannot be avoided, one would hypothesize that ants efficiently discriminate tapeworm-infected workers and furthermore employ behaviours such as rejection, physical avoidance or isolation of sick group members (e.g. Cremer et al. 2007). Non-volatile hydrocarbon profiles play a crucial role in the discrimination process of social insects. The composition of hydrocarbons informs social insects about the colony identity, the task and the health status of the encounter individual (e.g. Otto et al. 2018). Parasites commonly induce changes in the chemical profile of social

insects (e.g. Salvy et al. 2001; Baracchi et al. 2012). I find several enzymes involved in the synthesis of hydrocarbons to be differently expressed in tapeworm-infected workers, which indicates that the altered cuticular chemistry is the result of physiological changes (Chapter 3). The modified signature can be used by nestmates to identify sick individuals and to perform appropriate actions (e.g. sanitary care, aggression or elimination). Tapeworm-infected workers carry aberrant chemical profiles that provoke aggression in their nestmates and non-nestmates, but their profiles are also very similar to those of nurses, while the latter show much greater chemical differences to foragers (Chapter 2). A high similarity in recognition profiles makes it more likely that ants make mistakes, which can be costly if those errors lead to the aggression or elimination of healthy nestmates. Because tapeworm-infected workers share all cues with nestmates, this does not allow for a more accurate discrimination and explains why tapeworm-infected ant workers are not actively rejected or avoided in a social context, or excluded from the socially shared food (Chapter 1; 2; 4 & 5) – on the contrary. The occasional aggression towards tapeworm-infected workers would suggest that parasitized colonies may be generally more aggressive. Interestingly, healthy nestmates reduce their aggression towards non-nestmates, but continue to show high aggression against workers of the congeneric *Temnothorax affinis* (Chapter 1). The reduced aggression exclusively towards conspecifics may ultimately promote the tolerance of tapeworm-infected workers, but could also loosen the colony's cohesiveness and allow unrelated conspecifics to have access to colony resources (see e.g. Csata et al. 2017). The behavioural changes are directly linked to the presence of tapeworm-infected workers as these effects can be easily induced or reversed by introducing or removing tapeworm-infected workers (Chapter 1). Lower levels of aggression are often supposed to be a direct consequence of impaired recognition abilities due to relaxed identity of colony cues (Csata et al. 2017). However, I show that tapeworm-infected workers do not increase the chemical diversity within their colony (Chapter 2). Hence, behavioural changes cannot be attributed to a broader recognition template and must be shaped by other mechanisms (Chapter 2).

Part II - Conclusion and outlook

The co-evolutionary arms race between parasites and hosts results in counter-adaptations that help both biotic partners to reduce each other's costs and to maximize fitness. I present evidence that nestmates of tapeworm-infected workers are somehow triggered to care for their sick nestmates and that colony resources are therefore also directed towards the tapeworm's growth and survival (Chapter 4 & 5). These findings strongly indicate that the tapeworm *A. brevis* relies on its social host to remain inside the nest to complete its life cycle. If this is true, *A. brevis* has to ensure that its single ant host is not isolated from colony resources. In order to achieve this, *A. brevis* seems to be able to extend its manipulation to nestmates of its social host to secure its own survival (Chapter 1). My study system is ideal to study potential colony-wide manipulation and offers future research the possibility to explore, whether manipulation by parasites operates on multiple levels in (eu)-

social animals (e.g. Hughes et al. 2012). Work in this thesis has also identified potential costs at the social level. One assumption is that the high social investment in tapeworm-infected workers explains the reduced life expectancy of nestmates. Astonishingly, ants of *T. nylanderi* have obviously not evolved effective strategies to resist the infection. Observations rather suggest that ants seem to tolerate infected ants and compensate for the negative impact by employing yet unknown mechanisms. A lack of strong behavioural defences (i.e. rejection and aggression) is also observed in another co-evolved host-parasite association. Tropical carpenter ants serve as hosts for fungal species from the genus *Ophiocordyceps*. Although the parasite constitutes a constant threat and ultimately kills workers, infected foragers are allowed inside the colony and are neither attacked nor socially avoided (Loreto et al. 2014; Garcia et al. 2017). Future projects should investigate, if ants have evolved to be more tolerant, which could allow them to cope with losses of nestmates, without necessarily diminishing parasite fitness (e.g. Avilés 2017; Cremer et al. 2018).

References

Adamo, S.A., Robert, D., & Hoy, R.R. (1995). Effects of a tachinid parasitoid, *Ormia ochracea*, on the behaviour and reproduction of its male and female field cricket hosts (*Gryllus spp*.). *Journal of Insect Physiology*, 41, 269–277.

Adamo, S.A. (2013). Parasites: evolution's neurobiologists. *The Journal of Experimental Biology*, 216, 3–10.

Aeby, G.S. (1992). The potential effect the ability of a coral intermediate host to regenerate has had on the evolution of its association with a marine parasite. In *Proc. 7th Int. Coral Reef Symp. Guam*, 2, 809-815.

Aeby, G.S. (2002). Trade-offs for the butterflyfish, *Chaetodon multicinctus*, when feeding on coral prey infected with trematode metacercariae. *Behavioral Ecology and Sociobiology*, 52, 158-165.

Aeby, G.S. (2003). Corals in the genus *Porites* are susceptible to infection by a larval trematode. *Coral Reefs*, 22, 216-216.

Agnew, P., Koella, J.C., & Michalakis, Y. (2000). Host life history responses to parasitism. *Microbes and Infection*, 2, 891-896.

Ahmed, A.M., Baggott, S.L., Maingon, R., & Hurd, H. (2002). The costs of mounting an immune response are reflected in the reproductive fitness of the mosquito *Anopheles gambiae*. *Oikos*, 97, 371–377.

Akino, T., Yamamura, K., Wakamura, S., & Yamaoka, R. (2004). Direct behavioral evidence for hydrocarbons as nestmate recognition cues in *Formica japonica* (Hymenoptera: Formicidae). *Applied Entomology and Zoology*, 39, 381–387.

Alloway, T.M., Buschinger, A., Talbot, M., Stuart, R., & Thomas, C. (1982). Polygyny and polydomy in three North American species of the ant genus *Leptothorax* Mayr (Hymenoptera: Formicidae). *Psyche,* 89, 249-274.

Almberg, E.S., Cross, P.C., Dobson, A.P., Smith, D.W., Metz, M.C., Stahler, D.R., & Hudson, P.J. (2015). Social living mitigates the costs of a chronic illness in a cooperative carnivore. *Ecology Letters*, 18, 660–667.

Altschul, S.F., Gish, W., Miller, W., Myers, E.W., & Lipman, D.J. (1990). Basic local alignment search tool. *Journal of Molecular Biology*, 215, 403–410.

Amat, F., Gozalbo, A., Navarro, J. C., Hontoria, F., & Varó, I. (1991). Some aspects of *Artemia* biology affected by cestode parasitism. In D. Belk, H.J. Dumont & N. Munuswamy (Eds.), *Studies on Large Branchiopod Biology and Aquaculture. Developments in Hydrobiology,* (pp. 39-44).

Amdam, G. V. (2011). Social context, stress, and plasticity of aging. *Aging Cell*, 10, 18-27.

Andersen, S.B., Gerritsma, S., Yusah, K.M., Mayntz, D., Hywel-Jones, N.L., Billen, J., Boomsma, J.J., & Hughes, D.P. (2009). The life of a dead ant: the expression of an adaptive extended phenotype. *The American Naturalist,* 174, 424-433.

Aubert, A., & Richard, F.J. (2008). Social management of LPS-induced inflammation in *Formica polyctena* ants. *Brain, Behavior and Immunity*, 22, 833– 837.

Auld, S.K., & Tinsley, M.C. (2015). The evolutionary ecology of complex lifecycle parasites: linking phenomena with mechanisms. *Heredity,* 114, 125.

Avilés, J.M. (2017). Can hosts tolerate avian brood parasites? An appraisal of mechanisms. *Behavioral Ecology*, 29, 509-519.

Bakker, T.C., Mazzi, D., & Zala, S. (1997). Parasite-induced changes in behavior and color make *Gammarus pulex* more prone to fish predation. *Ecology*, 78, 1098-1104.

Baldauf, S.A., Thünken, T., Frommen, J.G., Bakker, T.C., Heupel, O., & Kullmann, H. (2007). Infection with an acanthocephalan manipulates an amphipod's reaction to a fish predator's odours. *International Journal for Parasitology,* 37, 61-65.

Baracchi, D., Fadda, A., & Turillazzi, S. (2012). Evidence for antiseptic behaviour towards sick adult bees in honey bee colonies. *Journal of Insect Physiology*, 58, 1589–1596.

Barnes, A. I., & Siva-Jothy, M. T. (2000). Density–dependent prophylaxis in the mealworm beetle *Tenebrio molitor L*. (Coleoptera: Tenebrionidae): cuticular melanization is an indicator of investment in immunity. *Proceedings of the Royal Society of London B: Biological Sciences*, 267, 177-182.

Barribeau, S.M., Sadd, B.M., du Plessis, L., & Schmid-Hempel, P. (2014). Gene expression differences underlying genotype-by-genotype specificity in a host-parasite system. *Proceedings of the National Academy of Sciences of the USA*, 111, 3496–3501.

Bates, D., Maechler, M., & Bolker, B. (2012). lme4: linear mixed-effects models using S4 classes. R package version 0.999999–0.

Baudoin, M. (1975). Host castration as a parasitic strategy. *Evolution,* 29, 335-352.

Bauer, S., Böhm, M., Witte, V., & Foitzik, S. (2010). An ant social parasite inbetween two chemical disparate host species. *BMC Evolutionary Biology*, 24, 317–332.

Beani, L. (2006). Crazy wasps: when parasites manipulate the *Polistes* phenotype. *Annales Zoologici Fennici*, 43, 564-574.

Beani, L., & Massolo, A. (2007). *Polistes dominulus* wasps (Hymenoptera Vespidae) if parasitized by *Xenos vesparum* (Strepsiptera Stylopidae) wander among nests during the pre-emerging phase. *Redia*, 90, 161-164.

Beani, L., Dallai, R., Mercati, D., Cappa, F., Giusti, F., & Manfredini, F. (2011). When a parasite breaks all the rules of a colony: morphology and fate of wasps infected by a strepsipteran endoparasite. *Animal Behaviour*, 82, 1305-1312.

Beckage, N.E. (1997). *New insights: how parasites and pathogens alter the endocrine physiology and development of insect hosts*. In N.E. Beckage (Eds). *Parasites and Pathogens. Effects on Hormons and Behaviour* (pp 3-36).

Benjamini, Y., Hochberg, Y., & Benjamini, Y. (1995). Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society Series B (Methodological)*, 57, 289–300.

Benesh, D.P., & Valtonen, E.T. (2007). Effects of *Acanthocephalus lucii* (Acanthocephala) on intermediate host survival and growth: implications for exploitation strategies. *Journal of Parasitology*, 93, 735-741.

Benesh, D.P., Valtonen, E.T., & Seppälä, O. (2008). Multidimensionality and intra-individual variation in host manipulation by an acanthocephalan. *Parasitology,* 135, 617-626.

Benesh, D. P., Chubb, J. C., & Parker, G. A. (2014). The trophic vacuum and the evolution of complex life cycles in trophically transmitted helminths. *Proceedings of the Royal Society of London B: Biological Sciences*, 281, 20141462.

Ben-Shahar, Y. (2005). The *foraging* gene, behavioral plasticity, and honeybee division of labor. *Journal of Comparative Physiology A*, 191, 987–994.

Berdoy, M., Webster, J.P., & Macdonald, D.W. (2000). Fatal attraction in rats infected with *Toxoplasma gondii*. *Proceedings of the Royal Society of London B: Biological Sciences*, 267, 1591-1594.

Berenreiterová, M., Flegr, J., Kuběna, A.A., & Němec, P. (2011). The distribution of *Toxoplasma gondii* cysts in the brain of a mouse with latent toxoplasmosis: implications for the behavioral manipulation hypothesis. *PloS One*, 6, e28925.

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 of London B: Biological Sciences*, 282, 1473.

Beros, S., Foitzik, S., & Menzel, F. (2017). What are the mechanisms behind a parasite-induced decline in nestmate recognition in ants? *Journal of Chemical Ecology*, 43, 869-880.

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*. In print.

Beros, S., Scharf, I., Lenhart, A., Negroni, M.A., Menzel, F., & Foitzik, S. Extreme lifespan extension in tapeworm-infected ants facilitated by increased care and an upregulation of longevity genes. Manuscript, submitted.

Bethel, W.M., & Holmes, J.C. (1973). Altered evasive behavior and responses to light in amphipods harboring acanthocephalan cystacanths. *The Journal of Parasitology*, 945-956.

Beye, M., Neumann, P., Chapuisat, M., Pamilo, P., & Moritz, R.F.A. (1998). Nestmate recognition and the genetic relatedness of nests in the ant *Formica pratensis*. *Behavioral Ecology and Sociobiology,* 43, 67–72.

Biron, D.G., Marché, L., Ponton, F., Loxdale, H.D., Galéotti, N., Renault, L., Joly, C., & Thomas, F. (2005). Behavioural manipulation in a grasshopper harbouring hairworm: a proteomics approach. *Proceedings of the Royal Society of London B: Biological Sciences,* 272, 2117–2126.

Biron, D.G., & Loxdale, H.D. (2013). Host-parasite molecular cross-talk during the manipulative process of a host by its parasite. *The Journal of Experimental Biology*, 216, 148–160.

Blanchard, G. B., Orledge, G. M., Reynolds, S. E., & Franks, N. R. (2000). Division of labour and seasonality in the ant *Leptothorax albipennis*: worker corpulence and its influence on behaviour. *Animal Behaviour*, 59, 723-738.

Blomquist, G.J., & Bagnères, A.G. (2010). Insect Hydrocarbons Biology, Biochemistry and Ecology. *Cambridge University Press*, Cambridge, UK.

Bocher, A., Doums, C., Millot, L., & Tirard, C. (2008). Reproductive conflicts affect labor and immune defense in the queenless ant *Diacamma* sp.*"nilgiri"*. *Evolution*, 62, 123-134.

Bodyak, N., Kang, P.M., Hiromura, M., Sulijoadikusumo, I., Horikoshi, N., Khrapko, K., & Usheva, A. (2002). Gene expression profiling of the aging mouse cardiac myocytes. *Nucleic Acid Research*, 30, 3788–3794.

Bolton, B. (2003). Synopsis and classification of Formicidae. *American Entomological Institute*, 71, 370.

Boomsma, J.J., Schmid-Hempel, P., & Hughes, W.O.H. (2005). Life histories and parasite pressure across the major groups of social insects. In M.D.E. Fellowes, G. Holloway & J. Rolff (Eds). *Insect Evolutionary Ecology* (pp. 139–76).

Bonavita-Cougourdan, A., Clement, J.L., & Lange, C. (1993). Functional subcaste discrimination (foragers and brood-tenders) in the ant *Camponotus vagus* scop: polymorphism of cuticular hydrocarbon patterns. *Journal of Chemical Ecology*, 19, 1461–1477.

Bos. N., Lefèvre. T., Jensen, A.B., & D'Ettorre, P. (2012). Sick ants become unsociable. *Journal of Evolutionary Biology*, 25, 342–351.

Botnevik, C.F., Malagocka, J., Jensen, A.B., & Fredensborg, B.L. (2016). Relative effects of temperature light and humidity on clinging behavior of metacercariae-infected ants. *Journal of Parasitology,* 102, 495-500.

Boulay, R., Hefetz, A., Soroker, V., & Lenoir, A. (2000). *Camponotus fellah* colony integration: worker individuality necessitates frequent hydrocarbon exchanges. *Animal Behaviour*, 59, 1127–1133.

Bourke, A.F. (1988). Worker reproduction in the higher eusocial Hymenoptera. *The Quarterly Review of Biology*, 63, 291-311.

Brandt, M., Heinze, J., Schmitt, T., & Foitzik, S. (2005). A chemical level in the coevolutionary arms race between an ant social parasite and its hosts. *Journal of Evolutionary Biology*, 18, 576–586.

Brown, M.J.F., Loosli, R., & Schmid-Hempel, P. (2000). Condition-dependent expression of virulence in a trypanosome infecting bumblebees. *Oikos*, 91, 421-427.

Brown, S.P., Renaud, F., Guégan, J.F., & Thomas, F. (2001). Evolution of trophic transmission in parasites: the need to reach a mating place? *Journal of Evolutionary Biology*, 14, 815-820.

Brown, S.P., Hochberg, M.E., & Grenfell, B.T. (2002). Does multiple infection select for raised virulence? *Trends in Microbiology*, 10, 401-405.

Brunner, E., & Heinze, J. (2009). Worker dominance and policing in the ant *Temnothorax unifasciatus*. *Insectes Sociaux,* 56, 397.

Bruschi, F., & Chiumiento, L. (2011). *Trichinella* inflammatory myopathy: host or parasite strategy? *Parasites & Vectors*, 4, 42.

Buechel, S.D., & Schmid-Hempel, P. (2016). Colony pace: a life-history trait affecting social insect epidemiology. *Proceedings of the Royal Society of London B: Biological Sciences*, 283, 20151919.

Buschinger, A. (1968). Mono- und Polygynie bei Arten der Gattung *Leptothorax Mayr* (Hymenoptera Formicidae). *Insectes Sociaux*, 15, 217-225.

Cameron, R. C., Duncan, E. J., & Dearden, P. K. (2013). Biased gene expression in early honeybee larval development. *BMC Genomics*, 14, 903.

Carney, W.P. (1969). Behavioral and morphological changes in carpenter ants harboring dicrocoeliid metacercariae. *American Midland Naturalist*, 82, 605–611.

Cézilly, F., & Perrot-Minnot, M.J. (2005). Studying adaptive changes in the behaviour of infected hosts: a long and winding road. *Behavioural Processes*, 68, 223-228.

Cézilly, F., & Perrot-Minnot, M.J. (2010). Interpreting multidimensionality in parasite-induced phenotypic alterations: panselectionism versus parsimony. *Oikos*, 119, 1224-1229.

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, 311–317.

Cézilly, F., Favrat, A., & Perrot-Minnot, M.J. (2013). Multidimensionality in parasite-induced phenotypic alterations: ultimate versus proximate aspects. *Journal of Experimental Biology*, 216, 27-35.

Cézilly, F., Perrot-Minnot, M.J., & Rigaud, T. (2014). Cooperation and conflict in host manipulation: interactions among macro-parasites and micro-organisms. *Frontiers in Microbiology,* 5, 248.

Chapuisat, M., Oppliger, A., Magliano, P., & Christe, P. (2007). Wood ants use resin to protect themselves against pathogens. *Proceedings of the Royal Society of London B: Biological Sciences*, 274, 2013-2017.

Charbonneau, D., Hillis, N., & Dornhaus, A. (2015). 'Lazy' in nature: ant colony time budgets show high 'inactivity' in the field as well as in the lab. *Insectes Sociaux*, 62, 31-35.

Charbonneau, D., Poff, C., Nguyen, H., Shin, M.C., Kierstead, K., & Dornhaus, A. (2017). Who are the "lazy" ants? The function of inactivity in social insects and a possible role of constraint: inactive ants are corpulent and may be young and/or selfish. *Integrative and Comparative Biology,* 57, 649-667.

Chen, Q., Niu, Y., Zhang, R., Guo, H., Gao, Y., Li, Y., & Liu, R. (2010). The toxic influence of paraquat on hippocampus of mice: involvement of oxidative stress. *Neurotoxicology*, 31, 310-316.

Chevreux, B., Wetter, T., & Suhai, S. (1999). Genome sequence assembly using trace signals and additional sequence information. *Computer Science and Biology: Proceedings of the German Conference on Bioinformatics*, 99, 45–56.

Choisy, M., Brown, S.P., Lafferty, K.D., & Thomas, F. (2003). Evolution of trophic transmission in parasites: why add intermediate hosts? *The American Naturalist*, 162, 172-181.

Christe, P., Oppliger, A., Bancalà, F., Castella, G., & Chapuisat, M. (2003). Evidence for collective medication in ants. *Ecology Letters*, 6, 19-22.

Cini, A., Gioli, L., & Cervo, R. (2009). A quantitative threshold for nest-mate recognition in a paper social wasp. *Biology Letters*, 5, 459–461.

Conesa, A., Götz, S., Garcia-Gómez, J.M., Terol, J., Talón, M., & Robles, M. (2005). Blast2GO: a universal tool for annotation, visualization and analysis in functional genomics research. *Bioinformatics*, 21, 3674–3676.

Cole, B.J. (1981). Dominance hierarchies in *Leptothorax* ants. *Science*, 212, 83-84.

Cole, B.J. (1986). The social behavior of *Leptothorax allardycei* (Hymenoptera, Formicidae): time budgets and the evolution of worker reproduction. *Behavioral Ecology and Sociobiology,* 18, 165-173.

Corbett, M.A., Robinson, C.S., Dunglison, G.F., Yang, N., Joya, J.E., Stewart, A.W., Schnell, C., Gunning, P.W., North, K.N., & Hardeman, E.C. (2001). A mutation in α-tropomyosin slow affects muscle strength, maturation and hypertrophy in a mouse model for nemaline myopathy. *Human Molecular Genetics*, 10, 317–328.

Cornet, S., Nicot, A., Rivero, A., & Gandon, S. (2013). Malaria infection increases bird attractiveness to uninfected mosquitoes. *Ecology Letters*, 16, 323-329.

Cotter, S. C., Hails, R. S., Cory, J. S., & WIlson, K. (2004). Density-dependent prophylaxis and condition-dependent immune function in Lepidopteran larvae: a multivariate approach. *Journal of Animal Ecology*, 73, 283-293.

Cremer. S., Armitage, S.A.O., & Schmid-Hempel, P. (2007). Social immunity. *Current Biology*, 17, 693-702.

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.

Crozier, R., & Dix, M.W. (1979). Analysis of two genetic models for the innate components of colony odor in social hymenoptera. *Behavioral Ecology and Sociobiology*, 4, 217–224.

Csata, E., Timus, N., Witek, M., Casacci, L.P., Lucas, C., Bagnères, A.G., Sztencel-Jablonka, A., Barbero, F., Bonelli, S., Rákosy, L., & Markó, B. (2017). Lock-picks: fungal infection facilitates the intrusion of strangers into ant colonies. *Scientific Reports*, 7, 46323.

Cuvillier-Hot, V., Cobb, M., Malosse, C., & Peeters, C. (2001). Sex, age and ovarian activity affect cuticular hydrocarbons in *Diacamma ceylonense*, a queenless ant. *Journal of Insect Physiology*, 47, 485–493.

Dani, F.R., Jones, G.R., Destri, S., Spencer, S.H., & Turillazzi, S. (2001). Deciphering the recognition signature within the cuticular chemical profile of paper wasps. *Animal Behaviour*, 62, 165–171.

Dani, F.R., Jones, G.R., Corsi, S., Beard, R., Pradella, D., & Turillazzi, S. (2005). Nestmate recognition cues in the honey bee: differential importance of cuticular alkanes and alkenes. *Chemical Senses*, 30, 477–489.

Dantzer, R., Connor, J.C.O., Freund, G.G., Johnson, R.W., & Kelley, K.W. (2008). From inflammation to sickness and depression: when the immune system subjugates the brain. *Nature*, 9, 46–57.

Dapporto, L., Cini, A., Palagi, E., Morelli, M., Simonti, A., & Turillazzi, S. (2007). Behaviour and chemical signature of pre-hibernating females of *Polistes dominulus* infected by the strepsipteran *Xenos vesparum*. *Parasitology,* 134, 545–552.

Davis, W.L., Jacoby, B.H., Jones, R.G., & Goodman, D.B. (1993). Superoxide formation preceding flight muscle histolysis in *Solenopsis*: fine structural cytochemistry and biochemistry. *The Histochemical Journal*, 25, 478-490.

Davis, T.S., Crippen, T.L., Hofstetter, R.W., & Tomberlin, J.K. (2013). Microbial volatile emissions as insect semiochemicals. *Journal of Chemical Ecology*, 39, 840–859.

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

de Bekker, C., Quevillon, L.E., Smith, P.B., et al. (2014). Species-specific ant brain manipulation by a specialized fungal parasite. *BMC Evolutionary Biology*, 14, 166.

de Bekker, C., Ohm, R.A., Loreto, R.G., Sebastian, A., Albert, I., Merrow, M., Brachmann, A., & Huhges, D.P. (2015). Gene expression during zombie ant biting behavior reflects the complexity underlying fungal parasitic behavioral manipulation. *BMC Genomics*, 16, 620.

de Bekker, C., Will, I., Das, B., & Adams, R.M.M. (2018). The ants (Hymenoptera: Formicidae) and their parasites: effects of parasitic manipulations and host responses on ant behavioral ecology. *Myrmecological News*, 28.

De Moraes, C.M., Stanczyk, N.M., Betz, H.S., Pulido, H., Sim, D.G., Read, A.F., & Mescher, M.C. (2014). Malaria-induced changes in host odors enhance mosquito attraction. *Proceedings of the National Academy of Sciences of the USA*, 111, 11079–11084.

Dheilly, N.M., Maure, F., Ravallec, M., Galinier, R., Doyon, J., Duval, D., Leger, L., Volkoff, A.N., Missé, D., Nidelet, S., Demolombe, V., Brodeur, J., Gourbal, B., Thomas, F., & Mitta, G. (2015). Who is the puppet master? Replication of a parasitic wasp-associated virus correlates with host behaviour manipulation. *Proceedings of the Royal Society of London B: Biological Sciences*, 282, 20142773.

Dianne, L., Perrot-Minnot, M.J., Bauer, A., Gaillard, M., Léger, E., & Rigaud, T. (2011). Protection first then facilitation: a manipulative parasite modulates the vulnerability to predation of its intermediate host according to its own developmental stage. *Evolution*, 65, 2692-2698.

Di Mauro, G., Perez, M., Lorenzi, M.C., Guerrieri, F.J., Millar, J.G., & D'Ettorre, P. (2015). Ants discriminate between different hydrocarbon concentrations. *Frontiers in Ecology and Evolution*, 3, 133.

Dietemann, V., Peeters, C., Liebig, J., Thivet, V., & Hölldobler, B. (2003). Cuticular hydrocarbons mediate discrimination of reproductive and nonreproductives in the ant *Myrmecia gulosa*. *Proceedings of the National Academy of Sciences of the USA,* 100, 10341–10346.

Diez, L., Deneubourg, J.L., & Detrain, C. (2012). Social prophylaxis through distant corpse removal in ants. *Naturwissenschaften*, 99, 833-842.

Diez, L., Lejeune, P., & Detrain, C. (2014). Keep the nest clean: survival advantages of corpse removal in ants. *Biology Letters*, 10, 20140306.

Dijkstra, M.B., & Boomsma, J.J. (2007). The economy of worker reproduction in *Acromyrmex* leafcutter ants. *Animal Behaviour*, 74, 519-529.

Dixon, L., Kuster, R., & Rueppell, O. (2014). Reproduction, social behavior, and aging trajectories in honeybee workers. *Age*, 36, 89-101.

Dobson, A.P., Hudson, P.J., & Lyles, A.M. (1992). Macroparasites: it's a wormy world. Crawley MJ, In Natural enemies. The population biology of predators parasites and diseases. *Blackwell Scientific Publications*, Oxford, UK.

Dobson, A.P., Lafferty, K.D., Kuris, A.M., Hechinger, R.F., & Jetz, W. (2008). Homage to Linnaeus: how many parasites? How many hosts? *Proceedings of the National Academy of Sciences*, 105, 11482-11489.

Dornhaus, A., Holley, J.A., Pook, V.G., Worswick, G., & Franks, N.R. (2008). Why do not all workers work? Colony size and workload during emigrations in the ant *Temnothorax albipennis*. *Behavioral Ecology and Sociobiology*, 63, 43-51.

Dornhaus, A., Holley, J.A., & Franks, N.R. (2009). Larger colonies do not have more specialized workers in the ant *Temnothorax albipennis*. *Behavioral Ecology*, 20, 922-929.

Durieux, R., Rigaud, T., & Médoc, V. (2012). Parasite-induced suppression of aggregation under predation risk in a freshwater amphipod: sociality of infected amphipods. *Behavioural Processes,* 91, 207-213

Eberhard, W.G. (2000). Spider manipulation by a wasp larva. *Nature*, 406, 255–256.

Ebert, D., Joachim Carius, H., Little, T., & Decaestecker, E. (2004). The evolution of virulence when parasites cause host castration and gigantism. *The American Naturalist*, 164, S19-S32.

Elia, M., Khalil, A., Bagnères, A. G., & Lorenzi, M. C. (2018). Appeasing their hosts: a novel strategy for parasite brood. *Animal Behaviour*, 146, 123-134.

Ellis, J.D., Hepburn, H.R., Ellis, A.M., & Elzen, P.J. (2003). Social encapsulation of the small hive beetle (*Aethina tumida Murray*) by European honeybees (*Apis mellifera* L.). *Insectes Sociaux*, 50, 286-291.

Errard, C. (1994). Development of interspecific recognition behavior in the ants *Manica rubida* and *Formica selysi* (Hymenoptera: Formicidae) reared in mixed-species groups. *Journal of Insect Behaviour*, 7, 83–99.

Errard, C., Hefetz, A., & Jaisson, P. (2006). Social discrimination tuning in ants: template formation and chemical similarity. *Behavioral Ecology and Sociobiology*, 59, 353–363.

Esponda, F., & Gordon, D.M. (2015). Distributed nestmate recognition in ants. *Proceedings of the Royal Society of London B: Biological Sciences*, 282, 20142838.

Evans, J. D., Aronstein, K., Chen, Y. P., Hetru, C., Imler, J. L., Jiang, H., Kanost, M., Thompson, G.J., Zou, Z., & Hultmark, D. (2006). Immune pathways and defence mechanisms in honey bees Apis mellifera. *Insect Molecular Biology*, 15, 645-656.

Evans, H.C., Elliot, S.L., & Hughes, D.P. (2011) Hidden diversity behind the zombie-ant fungus *Ophiocordyceps unilateralis*: four new species described from carpenter ants in Minas Gerais Brazil. *PloS One,* 6, e17024.

Feldmeyer, B., Elsner, D., & Foitzik, S. (2014). Gene expression patterns associated with caste and reproductive status in ants: worker-specific genes are more derived than queen-specific ones. *Molecular Ecology*, 23, 151–161.

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, 648–660.

Fenton, A., Magoolagan, L., Kennedy, Z., & Spencer, K.A. (2011). Parasite-induced warning coloration: a novel form of host manipulation. *Animal Behaviour*, 81, 417-422.

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

Foitzik, S., & Heinze, J. (1998). Nest site limitation and colony takeover in the ant *Leptothorax nylanderi*. *Behavioral Ecology*, 9, 367–375.

Foitzik, S., & Heinze, J. (2001). Microgeographic genetic structure and intraspecific parasitism in the ant *Leptothorax nylanderi*. *Ecological Entomology*, 26, 449–456.

Foitzik, S., Sturm, H., Pusch, K., D'Ettorre, P., & Heinze, J. (2007). Nestmate recognition and intraspecific chemical and genetic variation in *Temnothorax* ants. *Animal Behaviour*, 73, 999–1007.

Folch, J., Lees, M., & Sloane Stanley, G. H. (1957). A simple method for the isolation and purification of total lipides from animal tissues. *Journal of Biological Chemistry*, 226, 497-509.

Franceschi, N., Rigaud, T., Moret, Y., Hervant, F., & Bollache, L. (2007). Behavioural and physiological effects of the trophically transmitted cestode parasite *Cyathocephalus truncatus* on its intermediate host *Gammarus pulex*. *Parasitology*, 134, 1839-1847.

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, 1161-1170.

Franklin, E. L., Robinson, E. J., Marshall, J. A., Sendova-Franks, A. B., & Franks, N. R. (2012). Do ants need to be old and experienced to teach?. *Journal of Experimental Biology*, 215, 1287-1292.

Fredensborg, B. L., & Poulin, R. (2006). Parasitism shaping host life-history evolution: adaptive responses in a marine gastropod to infection by trematodes. *Journal of Animal Ecology*, 75, 44-53.

Fürst, M.A., Durey, M., & Nash, D.R. (2011). Testing the adjustable threshold model for intruder recognition on *Myrmica* ants in the context of a social parasite. *Proceedings of the Royal Society of London B: Biological Sciences*, 279, 516–522.

Gabrion, C., Plateaux, L., Quentin, C., & Barcelo, F. (1976). *Anomotaenia brevis* (Clerc, 1902) Fuhrmann, 1908, Cestode Cyclophyllide, parasite de *Leptothorax nylanderi* (Forster) Hyménoptère, Formicidé. *Annales de parasitologie humaine et ompare,* 51, 407-420.

Geffre, A.C., Liu, R., Manfredini, F., Beani, L., Kathirithamby, J., Grozinger, C.M., & Toth, A.L. (2017). Transcriptomics of an extended phenotype: parasite manipulation of wasp social behaviour shifts expression of caste-related genes. *Proceedings of the Royal Society of London B: Biological Sciences*, 284, 20170029.

Geiser, D. L., & Winzerling, J. J. (2012). Insect transferrins: multifunctional proteins. *Biochimica et Biophysica Acta (BBA)-General Subjects*, 1820, 437-451.

Gibbs, A. (1995). Physical properties of insect cuticular hydrocarbons: model mixtures and lipid interactions. *Comparative Biochemistry and Physiology Part B*, 112, 667–672.

Gibbs, A., & Pomonis, J.G. (1995). Physical properties of insect cuticular hydrocarbons: the effects of chain length, methyl-branching and unsaturation. *Comparative Biochemistry and Physiology Part B*, 112, 243–249.

Giehr, J., Grasse, A.V., Cremer, S., Heinze, J., & Schrempf, A. (2017). Ant queens increase their reproductive efforts after pathogen infection. *Royal Society open science*, 4, 170547.

Gobin, B., Heinze, J., Strätz, M., & Roces, F. (2003). The energetic cost of reproductive conflicts in the ant *Pachycondyla obscuricornis*. *Journal of Insect Physiology,* 49, 747-752.

Goodman, B.A., & Johnson, P.T. (2011). Disease and the extended phenotype: parasites control host performance and survival through induced changes in body plan. *PLoS One*, 6, e20193.

Gopko, M., Mikheev, V. N., & Taskinen, J. (2015). Changes in host behaviour caused by immature larvae of the eye fluke: evidence supporting the predation suppression hypothesis. *Behavioral Ecology and Sociobiology*, 69, 1723-1730.

Gracia, E.S., de Bekker, C., Hanks, E.M., & Hughes, D.P. (2018). Within the fortress: A specialized parasite is not discriminated against in a social insect society. *PloS One*, 13, e0193536.

Greco, M.K., Hoffmann, D., Dollin, A., Duncan, M., Spooner-Hart, R., & Neumann, P. (2010). The alternative Pharaoh approach: stingless bees mummify beetle parasites alive. *Naturwissenschaften*, 97, 319-323.

Greene, M.J., & Gordon, D.M. (2003). Social insects: cuticular hydrocarbons inform task decisions. *Nature*, 423, 32.

Grosman, A.H., Janssen, A., De Brito, E.F., Cordeiro, E.G., Colares, F., Fonseca, J.O., Lima, E.R., Pallini, A., & Sabelis, M.W. (2008). Parasitoid increases survival of its pupae by inducing hosts to fight predators. *Plos One*, 3, e2276.

Grozinger, C.M., Fan, Y., Hoover, S.E.R., & Winston, M.L. (2007). Genome-wide analysis reveals differences in brain gene expression patterns associated with caste and reproductive status in honey bees (*Apis mellifera*). *Molecular Ecology*, 16, 4837–4848.

Guerrieri, F.J., Nehring, V., Jørgensen, J.G., Nielsen, J., Galizia, C.G., & D'Ettorre, P. (2009). Ants recognize foes and not friends. *Proceedings of the Royal Society of London B: Biological Sciences,* 276, 2461– 2468.

Gunning, P., Neill, G.O., & Hardeman, E. (2008). Tropomyosin-based regulation of the actin cytoskeleton in time and space. *Physiological Reviews*, 88, 1–35.

Hafer, N., & Milinski, M. (2015a). When parasites disagree: evidence for parasite-induced sabotage of host manipulation. *Evolution,* 69, 611-620.

Hafer, N., & Milinski, M. (2015b). An experimental conflict of interest between parasites reveals the mechanism of host manipulation. *Behavioral Ecology*, 27, 617-627.

Hafer, N., & Milinski, M. (2016). Inter-and intraspecific conflicts between parasites over host manipulation. *Proceedings of the Royal Society of London B: Biological Sciences,* 283, 20152870.

Haine, E.R., Boucansaud, K., & Rigaud, T. (2005). Conflict between parasites with different transmission strategies infecting an amphipod host. *Proceedings of the Royal Society of London B: Biological Sciences,* 272, 2505-2510.

Hakimi, M., & Cannella, D. (2011). Apicomplexan parasites and subversion of the host cell microRNA pathway. *Trends in Parasitology*, 27, 481–486.

Hammerschmidt, K., & Kurtz, J. (2005). Surface carbohydrate composition of a tapeworm in its consecutive intermediate hosts: individual variation and fitness consequences. *International Journal for Parasitology*, 35, 1499-1507.

Hammerschmidt, K., Koch, K., Milinski, M., Chubb, J. C., & Parker, G. A. (2009). When to go: optimization of host switching in parasites with complex life cycles. *Evolution: International Journal of Organic Evolution*, 63, 1976-1986.

Hands, S.L., Proud, C.G., & Wyttenbach, A. (2009). mTOR's role in ageing: protein synthesis or autophagy? *Aging*, 1, 586–597.

Hari Dass, S.A., & Vyas, A. (2014). *Toxoplasma gondii* infection reduces predator aversion in rats through epigenetic modulation in the host medial amygdala. *Molecular Ecology*, 23, 6114–6122.

Hasegawa, E., Ishii, Y., Tada, K., Kobayashi, K., & Yoshimura, J. (2016). Lazy workers are necessary for long-term sustainability in insect societies. *Scientific Reports*, 6, 20846.

Hébert, F.O., Grambauer, S., Barber, I., Landry, C.R., & Aubin-Horth, N. (2017). Major host transitions are modulated through transcriptome-wide reprogramming

events in *Schistocephalus solidus*, a threespine stickleback parasite. *Molecular Ecology*, 26, 1118-1130.

Heinze, J., Foitzik, S., Hippert, A., & Hölldobler, B. (1996). Apparent dear enemy phenomenon and environment-based recognition cues in the ant *Leptothorax nylanderi*. *Ethology*, 102, 510–522.

Heinze, J., Puchinger, W., & Hölldobler, B. (1997). Worker reproduction and social hierarchies in *Leptothorax* ants. *Animal Behaviour,* 54, 849-864.

Heinze, J., Rüppell, O., Foitzik, S., & Buschinger, A. (1998). First records of *Leptothorax rugatulus* (Hymenoptera: Formicidae) with cysticercoids of tapeworms (Cestoda: Dilepididae) from the southwestern United States. *The Florida Entomologist*, 81, 122-125.

Heinze, J. (2008). Hierarchy length in orphaned colonies of the ant *Temnothorax nylanderi*. *Naturwissenschaften*, 95, 757-760.

Heinze, J., & Walter, B. (2010). Moribund ants leave their nests to die in social isolation. *Current Biology*, 20, 249-252.

Heinze, J., Frohschammer, S., & Bernadou, A. (2013). Queen life-span and total reproductive success are positively associated in the ant *Cardiocondyla cf. kagutsuchi*. *Behavioral Ecology and Sociobiology*, 67, 1555-1562.

Herbison, R., Lagrue, C., & Poulin, R. (2018). The missing link in parasite manipulation of host behaviour. *Parasites & Vectors*, 11, 222.

Høeg, J.T. (1995). The biology and life cycle of the *Rhizocephala* (Cirripedia). *Journal of the Marine Biological Association of the United Kingdom*, 75, 517-550.

Hohorst, W., & Graefe, G. (1961). Ameisen - obligatorische Zwischenwirte des Lanzettegels (*Dicrocoelium dendriticum*). *Naturwissenschaften*, 48, 229–230.

Holliday, R. (2006). Aging is no longer an unsolved problem in biology. *Annals of the New York Academy of Sciences*, 1067, 1–9.

Hoover, K., Grove, M., Gardner, M., Hughes, D.P., McNeil, J., & Slavicek, J. (2011). A gene for an extended phenotype. *Science*, 333, 1401-1401.

Hosamani, R., & Muralidhara. (2013). Acute exposure of *Drosophila melanogaster* to paraquat causes oxidative stress and mitochondrial dysfunction. *Archives of Insect Biochemistry and Physiology*, 83, 25-40.

Hughes, W.H.O., Eilenberg, J., & Boomsma, J.J. (2002). Trade-offs in group living: transmission and disease resistance in leaf-cutting ants. *Proceedings of the Royal Society of London B: Biological Sciences*, 269, 1811–1819.

Hughes, D.P., Kathirithamby, J., Turillazzi, S., & Beani, L. (2004). Social wasps desert the colony and aggregate outside if parasitized: parasite manipulation? *Behavioral Ecology*, 15, 1037-1043.

Hughes, D. P., Andersen, S. B., Hywel-Jones, N. L., Himaman, W., Billen, J., & Boomsma, J. J. (2011). Behavioral mechanisms and morphological symptoms of zombie ants dying from fungal infection. *BMC Ecology*, 11, 13.

Hughes, D.P., Brodeur, J., & Thomas, F. (2012). Host manipulation by parasites. *Oxford University Press.* Oxford, UK.

Hughes, D. (2013). Pathways to understanding the extended phenotype of parasites in their hosts. *Journal of Experimental Biology*, 216, 142-147.

Hughes, D.P., & Libersat, F. (2018). Neuroparasitology of parasite–insect associations. *Annual Review of Entomology*, 63, 471-487.

Hurd, H., & Fogo, S. (1991). Changes induced by *Hymenolepis diminuta* (Cestoda) in the behaviour of the intermediate host *Tenebrio molitor* (Coleoptera). *Canadian Journal of Zoology*, 69, 2291-2294.

Hurd, H. (2001). Host fecundity reduction: a strategy for damage limitation? *Trends in Parasitology*, 17, 363-368.

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

Hurd, H. (2003). Manipulation of medically important insect vectors by their parasites. *Annual Review of Entomology,* 48, 141-161.

Hussain, M., Frentiu, F.D., Moreira, L.A., O'Neill, S.L., & Asgari, S. (2011). *Wolbachia* uses host microRNAs to manipulate host gene expression and facilitate colonization of the dengue vector *Aedes aegypti*. *Proceedings of the National Academy of Sciences*, 108, 9250-9255.

Ibrahim, A., & Spivak, M. (2006). The relationship between hygienic behaviour and suppression of mite reproduction as honey bee (*Apis mellifera*) mechanisms of resistance to *Varroa destructor*. *Apidologie*, 37, 31–40.

Imhoof, B., & Schmid-Hempel, P. (1999). Colony success of the bumble bee, *Bombus terrestris*, in relation to infections by two protozoan parasites, *Crithidia bombi* and *Nosema bombi*. *Insectes Sociaux*, 46, 233-238.

Ingram, K.K., Oefner, P., & Gordon, D.M. (2005). Task-specific expression of the foraging gene in harvester ants. *Molecular Ecology*, 14, 813–818.

Johnson, S.C., Rabinovitch, P.S., & Kaeberlein, M. (2013). mTOR is a key modulator of ageing and age-related disease. *Nature*, 493, 338–345.

Jongepier, E., Kleeberg, I., & Foitzik, S. (2015). The ecological success of a social parasite increases with manipulation of collective host behaviour. *Journal of Evolutionary Biology,* 28, 2152-2162.

Jongepier, E., & Foitzik, S. (2016). Ant recognition cue diversity is higher in the presence of slavemaker ants. *Behavioral Ecology*, 27, 304–311.

Kaeberlein, M., Burtner, C.R., & Kennedy, B.K. (2007). Recent developments in yeast aging. *PLoS Genetics*, 3, e84.

Kahsai, L., Martin, J., & Winther, A.M.E. (2010). Neuropeptides in the *Drosophila* central complex in modulation of locomotor behavior. *The Journal of Experimental Biology*, 213, 2256–2265.

Kaldonski, N., Perrot-Minnot, M.J., Motreuil, S., & Cézilly, F. (2008). Infection with acanthocephalans increases the vulnerability of *Gammarus pulex* (Crustacea Amphipoda) to non-host invertebrate predators. *Parasitology*, 135, 627-632.

Kaldonski, N., Perrot-Minnot, M.J., Dodet, R., Martinaud, G., & Cézilly, F. (2009). Carotenoid-based colour of acanthocephalan cystacanths plays no role in host manipulation. *Proceedings of the Royal Society B: Biological Sciences*, 276, 169-176.

Kamiya, T., O'dwyer, K., Nakagawa, S., & Poulin, R. (2014). Host diversity drives parasite diversity: meta-analytical insights into patterns and causal mechanisms. *Ecography*, 37, 689-697.

Kather, R., Drijfhout, F.P., & Martin, S.J. (2011). Task group differences in cuticular lipids in the honey bee *Apis mellifera*. *Journal of Chemical Ecology*, 37, 205–212.

Katzav-Gozansky, T., Boulay, R., Ionescu-Hirsh, A., & Hefetz, A. (2008). Nest volatiles as modulators of nestmate recognition in the ant *Camponotus fellah*. *Journal of Insect Physiology*, 54, 378–385.

Keiser, C.N., Vojvodic, S., Butler, I.O., Sartain, E., Rudolf, V.H., & Saltz, J.B. (2018). Queen presence mediates the relationship between collective behaviour and disease susceptibility in ant colonies. *Journal of Animal Ecology,* 87, 379-387.

Keller, L., & Genoud, M. (1997). Extraordinary lifespans in ants: a test of evolutionary theories of ageing. *Nature*, 389, 958–960.

Keller, L. (1998). Queen lifespan and colony characteristics in ants and termites. *Insectes Sociaux*, 45, 235-246.

Kent, C.F., Daskalchuk, T., Cook, L., Sokolowski, M.B., & Greenspan, R.J. (2009). The *Drosophila* foraging gene mediates adult plasticity and gene-environment interactions in behaviour, metabolites, and gene expression in response to food deprivation. *PLoS Genetics*, 5, e1000609.

Kim, B. Y., Lee, K. S., Choo, Y. M., Kim, I., Je, Y. H., Woo, S. D., Lee, S.M., Park, H.C., Sohn, H.D., & Jin, B. R. (2008). Insect transferrin functions as an antioxidant protein in a beetle larva. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, 150, 161-169.

Kleeberg, I., Menzel, F., & Foitzik, S. (2017). The influence of slavemaking lifestyle, caste and sex on chemical profiles in *Temnothorax* ants: insights into the evolution of cuticular hydrocarbons. *Proceedings of the Royal Society of London B: Biological Sciences*, 284, 20162249.

Klein, S.L. (2003). Parasite manipulation of the proximate mechanisms that mediate social behavior in vertebrates. *Physiology & Behavior*, 79, 441–449.

Klichko, V., Sohal, B.H., Radyuk, S.N., Orr, W.C., & Sohal, R.S. (2014). Decrease in cytochrome c oxidase reserve capacity diminishes robustness of *Drosophila melanogaster* and shortens lifespan. *The Biochemical Journal*, 459, 127–135.

Koella, J.C., Rieu, L., & Paul, R.E. (2002). Stage-specific manipulation of a mosquito's host-seeking behavior by the malaria parasite *Plasmodium gallinaceum*. *Behavioral Ecology*, 13, 816-820.

Kohlmeier, P., Negroni, M.A., Kever, M., Emmling, S., Stypa, H., Feldmeyer, B., & Foitzik, S. (2017). Intrinsic worker mortality depends on behavioral caste and the queens' presence in a social insect. *The Science of Nature*, 104, 34.

Kohlmeier, P., Feldmeyer, B., & Foitzik, S. (2018a). *Vitellogenin-like A*–associated shifts in social cue responsiveness regulate behavioral task specialization in an ant. *PLoS Biology*, 16, e2005747.

Kohlmeier, P., Alleman, A. R., Libbrecht, R., Foitzik, S., & Feldmeyer, B. (2018b). Gene expression is more strongly associated with behavioural specialisation than with age or fertility in ant workers. *Molecular Ecology*. In print.

Konrad, M., Vyleta, M.L., Theis, F.J., Stock, M., Tragust, S., Klatt, M., Drescher, V., Marr, C., Ugelvig, L.V., & Cremer, S. (2012a). Social transfer of pathogenic fungus promotes active immunization in ant colonies. *PLoS Biology*, 10, e1001300.

Konrad, M., Pamminger, T., & Foitzik, S. (2012b). Two pathways ensuring social harmony. *Naturwissenschaften*, 99, 627–636.

Kristensen, T., Nielsen, A. I., Jørgensen, A. I., Mouritsen, K. N., Glenner, H., Christensen, J. T., Lützen, T., & Høeg, J. T. (2012). The selective advantage of host feminization: a case study of the green crab *Carcinus maenas* and the parasitic barnacle *Sacculina carcini*. *Marine Biology*, 159, 2015-2023.

Krull, W.H., & Mapes, C.R. (1953). Studies on the biology of *Dicrocoelium dendriticum* (Rudolphi 1819) Looss 1899 (Trematoda: Dicrocoeliidae) including its relation to the intermediate host *Cionella lubrica* (Müller). IX. Notes on the cyst metacercaria and infection in the ant *Formica fusca*. *Cornell Veterinarian*. 43, 389-410.

Kucharski, R., & Maleszka, R. (2003). Transcriptional profiling reveals multifunctional roles for transferrin in the honeybee, *Apis mellifera*. *Journal of Insect Science*, 3.

Kuszewska, K., Miler, K., & Woyciechowski, M. (2018). Honeybee rebel workers invest less in risky foraging than normal workers. *Scientific Reports*, 8, 9459.

König, C., & Schmid-Hempel, P. (1995). Foraging activity and immunocompetence in workers of the bumble bee, *Bombus terrestris* L. *Proceedings of the Royal Society of London B: Biological Sciences*, 260, 225-227.

Kühbandner, S., Modlmeier, A.P., & Foitzik, S. (2014). Age and ovarian development are related to worker personality and task allocation in the ant *Leptothorax acervorum*. *Current Zoology*, 60, 392-400.

Lafferty, K.D. (1999). The evolution of trophic transmission. *Parasitology Today,* 15, 111-115.

Lafferty, K. D., & Kuris, A. M. (2009). Parasitic castration: the evolution and ecology of body snatchers. *Trends in Parasitology*, 25, 564-572.

Lafferty, K.D., Shaw, J.C. (2013). Comparing mechanisms of host manipulation across host and parasite taxa. *Journal of Experimental Biology*, 216, 56-66.

Lahav, S., Soroker, V., Hefetz. A., Vander Meer, R.K. (1999). Direct behavioral evidence for hydrocarbons as ant recognition discriminators. *Naturwissenschaften*, 86, 246–249.

Lamberton, P.H.L., Donnelly, C.A., Webster, J.P. (2008). Specificity of the *Toxoplasma gondii*-altered behaviour to definitive versus non-definitive host predation risk. *Parasitology*, 135, 1143-1150.

Langfelder, P., Horvath, S. (2008). WGCNA: an R package for weighted correlation network analysis. *BMC Boinformatics*, 9, 559.

Larsen, J., Fouks, B., Bos, N., D'Ettorre, P., & Nehring, V. (2014). Variation in nestmate recognition ability among polymorphic leaf-cutting ant workers. *Journal of Insect Physiology*, 70, 59–66.

LeBoeuf, A.C., Waridel, P., Brent, C.S., Gonçalves, A.N., Menin, L., Ortiz, D., Riba-Grognuz, O., Koto, A., Soares, Z.G., Privman, E., Miska, E.A., Benton, R., & Keller, L. (2016). Oral transfer of chemical cues, growth proteins and hormones in social insects. *Elife*, 5, e20375.

Leclerc, J.B., & Detrain, C. (2017). Loss of attraction for social cues leads to fungal-infected *Myrmica rubra* ants withdrawing from the nest. *Animal Behaviour*, 129, 133-141.

Leclerc, J.B., & Detrain, C. (2018). Impact of colony size on survival and sanitary strategies in fungus-infected ant colonies. *Behavioral Ecology and Sociobiology*, 72, 3.

Lecocq, A., Jensen, A.B., Kryger, P., & Nieh, J.C. (2016). Parasite infection accelerates age polyethism in young honey bees. *Scientific Reports*, 6, 22042.

Lee, K.P., Cory, J.S., Wilson, K., Raubenheimer, D., & Simpson, S.J. (2006a). Flexible diet choice offsets protein costs of pathogen resistance in a caterpillar. *Proceedings of the Royal Society of London B: Biological Sciences*, 273, 823-82.

Lee, K. S., Kim, B. Y., Kim, H. J., Seo, S. J., Yoon, H. J., Choi, Y. S., Kim, I., Han, Y.S., Je, Y.H., Lee, S.M., Kim, D.H., Sohn, H.D., & Jin, B.R. (2006b). Transferrin inhibits stress-induced apoptosis in a beetle. *Free Radical Biology and Medicine*, 41, 1151-1161.

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, 435-439.

Lenoir, A., D'Ettorre, P., & Errard, C. (2001). Chemical ecology and social parasitism in ants. *Annual Review of Entomology*, 46, 573–599.

Leonhardt, S.D., Brandstaetter, A.S., & Kleineidam, C.J. (2007). Reformation process of the neuronal template for nestmate-recognition cues in the carpenter ant *Camponotus floridanus*. *Journal of Comparative Physiology A*, 193, 993–1000.

Leonhardt, S.D., Menzel, F., Nehring, V., & Schmitt, T. (2016). Ecology and evolution of communication in social insects. *Cell*, 164, 1277–1287.

Lesnoff, M., & Lancelot, R. (2012). aod: analysis of overdispersed data. R package version 1.3.

Liang, D., & Silverman, J. (2000). "You are what you eat": diet modifies cuticular hydrocarbons and nestmate recognition in the Argentine ant, *Linepithema humile*. *Naturwissenschaften*, 87, 412–416.

Libersat, F., Delago, A., & Gal, R. (2009). Manipulation of host behavior by parasitic insects and insect parasites. *Annual Review of Entomology*, 54, 189–207.

López, J.H., Riessberger-Gallé, U., Crailsheim, K., & Schuehly, W. (2017). Cuticular hydrocarbon cues of immune-challenged workers elicit immune activation in honeybee queens. *Molecular Ecology*, 26, 3062–3073.

Lopez-Vaamonde, C., Raine, N.E., Koning, J.W., Brown, R.M., Pereboom, J.J.M., Ings, T.C., Ramos-Rodriguez, O., Jordan, W.C., & Bourke, A.F.G. (2009). Lifetime reproductive success and longevity of queens in an annual social insect. *Journal of Evolutionary Biology,* 22, 983-996.

Loreto, R.G., Elliot, S.L., Freitas, M.L., Pereira, T.M., & Hughes, D.P. (2014). Long-term disease dynamics for a specialized parasite of ant societies: a field study. *PloS One*, 9, e103516.

Lucas, E. R., Privman, E., & Keller, L. (2016). Higher expression of somatic repair genes in long-lived ant queens than workers. *Aging*, 8, 1940.

Maitland, D.P. (1994). A parasitic fungus infecting yellow dungflies manipulates host perching behaviour. *Proceedings of the Royal Society of London B: Biological Sciences,* 258, 187-193.

Martin, S.J., Vitikainen, E., Helanterä, H., & Drijfhout, F.P. (2008). Chemical basis of nest-mate discrimination in the ant *Formica exsecta*. *Proceedings of the Royal Society of London B: Biological Sciences*, 275, 1271–1278.

Martin, S.J., & Drijfhout, F.P. (2009). Nestmate and task cues are influenced and encoded differently within ant cuticular hydrocarbon profiles. *Journal of Chemical Ecology*, 35, 368–374.

Martin, R. C., Vining, K., & Dombrowski, J. E. (2018). Genome-wide (ChIP-seq) identification of target genes regulated by BdbZIP10 during paraquat-induced oxidative stress. *BMC Plant Biology*, 18, 58.

Martín-Vega, D., Garbout, A., Ahmed, F., Wicklein, M., Goater, C.P., Colwell, D.D., & Hall, M.J. (2018). 3D virtual histology at the host/parasite interface: visualisation of the master manipulator, *Dicrocoelium dendriticum*, in the brain of its ant host. *Scientific Reports*, 8, 8587.

Mas, F., Haynes, K.F., & Kölliker, M. (2009). A chemical signal of offspring quality affects maternal care in a social insect. *Proceedings of the Royal Society of London B: Biological Sciences*, 276, 2847–2853.

Maure, F., Brodeur, J., Ponlet, N., Doyon, J., Firlej, A., Elguero, É., & Thomas, F. (2011). The cost of a bodyguard. *Biology Letters*, 7, 843-846.

McDonnell, C.M., Alaux, C., Parrinello, H., Desvignes, J.P., Crauser, D., Durbesson, E., Beslay, D., Le Conte, Y. (2013). Ecto- and endoparasite induce similar chemical and brain neurogenomic responses in the honey bee (*Apis mellifera*). *BMC Ecology*, 13, 25.

McDonough, K.A., & Rodriguez, A. (2012). The myriad roles of cyclic AMP in microbial pathogens: from signal to sword. *Nature Reviews Microbiology*, 10, 27–38.

Médoc, V., Rigaud, T., Bollache, L., & Beisel, J.N. (2009). A manipulative parasite increasing an antipredator response decreases its vulnerability to a nonhost predator. *Animal Behaviour,* 77, 1235-1241.

Mehdiabadi, N.J., & Gilbert, L.E. (2002). Colony–level impacts of parasitoid flies on fire ants. *Proceedings of the Royal Society of London B: Biological Sciences*, 269, 1695–1699.

Mersch, D.P., Crespi, A., & Keller, L. (2013). Tracking individuals shows spatial fidelity is a key regulator of ant social organization. *Science*, 340, 1090–1093.

Michalakis, Y. (2009). Parasitism and the evolution of life-history traits. In F. Thomas, J-F. Guégan & F. Renaud (Eds.), *Ecology and Evolution of Parasitism.* (pp. 19-30).

Minchella, D.J. (1985). Host life-history variation in response to parasitism. *Parasitology*, 90, 205-216.

Modlmeier, A.P., & Foitzik, S. (2011). Productivity increases with variation in aggression among group members in *Temnothorax* ants. *Behavior Ecology*, 22, 1026–1032.

Modlmeier, A.P., Pamminger, T., Foitzik, S., & Scharf, I. (2012). Cold resistance depends on acclimation and behavioral caste in a temperate ant. *Naturwissenschaften*, 99, 811–819.

Monnin, T., & Peeters, C. (1999). Dominance hierarchy and reproductive conflicts among subordinates in a monogynous queenless ant. *Behavioral Ecology*, 10, 323-332.

Moore, J., & Gotelli, N. J. (1990). A phylogenetic perspective on the evolution of altered host behaviours: A critical look at the manipulation hypothesis. *Parasitism and host behaviour*, 193-233.

Moore, J. (1995). The behavior of parasitized animals. *Bioscience*, 45, 89–96.

Moore, J. (2002). Parasites and the behavior of animals. *Oxford University Press*, Oxford, UK.

Morandin, C., Havukainen, H., Kulmuni, J., Dhaygude, K., Trontti, K., & Helanterä, H. (2014). Not only for egg yolk–functional and evolutionary insights from expression, selection, and structural analyses of *Formica* ant vitellogenins. *Molecular Biology and Evolution*, 31, 2181–2193.

Morel, L., Vander Meer, R.K., & Lofgren, C.S. (1990). Comparison of nestmate recognition between monogyne and polygyne populations of *Solenopsis invicta* (hymenoptera: Formicidae). *Annales of the Entomological Society of America*, 83, 642–647.

Moret, Y., & Schmid-Hempel, P. (2000). Survival for immunity: the price of immune system activation for bumblebee workers. *Science*, 290, 1166.

Moroń, D., Witek, M., & Woyciechowski, M. (2008). Division of labour among workers with different life expectancy in the ant *Myrmica scabrinodis*. *Animal Behaviour*, 75, 345-350.

Mouritsen, K.N. (2002). The parasite-induced surfacing behaviour in the cockle *Austrovenus stutchburyi*: a test of an alternative hypothesis and identification of potential mechanisms. *Parasitology*, 124, 521–528.

Mouritsen, K.N., & Poulin, R. (2003a). The risk of being at the top: foot-cropping in the New Zealand cockle *Austrovenus stutchburyi*. *Journal of the Marine Biological Association of the United Kingdom*, 83, 497-498.

Mouritsen, K.N., & Poulin, R. (2003b). Parasite-induced trophic facilitation exploited by a non-host predator: a manipulator's nightmare. *International Journal for Parasitology*, 33, 1043-1050.

Muir, D. A. (1954). Ants *Myrmica rubra L*. and *M. scabrinodis Nylander* as intermediate hosts of a cestode. *Nature*, 173, 688.

Natsopoulou, M.E., McMahon, D.P., & Paxton, R.J. (2016). Parasites modulate within-colony activity and accelerate the temporal polyethism schedule of a social insect, the honey bee. *Behavioral Ecology and Sociobiology*, 70, 1019-1031.

Naug, D., & Smith, B. (2007). Experimentally induced change in infectious period affects transmission dynamics in a social group. *Proceedings of the Royal Society of London B: Biological Sciences*, 274, 61-65.

Negroni, M.A., Jongepier, E., Feldmeyer, B., Kramer, B.H., & Foitzik, S. (2016). Life history evolution in social insects: a female perspective. *Current Opinion in Insect Science*, 16, 51-57.

Newey, P.S., Robson, S.K., & Crozier, R.H. (2010). Know thine enemy: why some weaver ants do but others do not. *Behavioral Ecology*, 21, 381– 386.

Newey, P. (2011). Not one odour but two: a new model for nestmate recognition. *Journal of Theoretical Biology*, 270, 7–12.

Nichol, H., Law, J. H., & Winzerling, J. J. (2002). Iron metabolism in insects. *Annual Review of Entomology*, 47, 535-559.

Nässel, D.R. (2002). Neuropeptides in the nervous system of *Drosophila* and other insects: multiple roles as neuromodulators and neurohormones. *Progress in Neurobiology*, 68, 1–84.

Nässel, D.R., & Winther, M.E. (2010). *Drosophila* neuropeptides in regulation of physiology and behavior. *Progress in Neurobiology*, 92, 42–104.

Okada, Y., Plateaux, L., & Peeters, C. (2013). Morphological variability of intercastes in the ant *Temnothorax nylanderi*: pattern of trait expression and modularity. *Insectes Sociaux*, 60, 319-328.

Otte, T., Hilker, M., & Geiselhardt, S. (2018). Phenotypic plasticity of cuticular hydrocarbon profiles in insects. *Journal of Chemical Ecology*, 44, 235-247.

Paily, K. P., Kumar, B. A., & Balaraman, K. (2007). Transferrin in the mosquito, *Culex quinquefasciatus Say* (Diptera: Culicidae), up-regulated upon infection and development of the filarial parasite, *Wuchereria bancrofti* (Cobbold)(Spirurida: Onchocercidae). *Parasitology Research*, 101, 325-330.

Palmer, C.V., Roth, M.S., & Gates, R.D. (2009). Red fluorescent protein responsible for pigmentation in trematode-infected *Porites compressa* tissues. *The Biological Bulletin*, 216, 68-74.

Pamminger, T., Modlmeier, A.P., Suette, S., Pennings, P.S., & Foitzik, S. (2012). Raiders from the sky: slavemaker founding queens select for aggressive host colonies. *Biology Letters*, 8, 748-750.

Pamminger, T., Foitzik, S., Kaufmann, K.C., Schützler, N., & Menzel, F. (2014). Worker personality and its association with spatially structured division of labor. *PLoS One*, 9, 1–8.

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

Parker, G. A., Ball, M. A., Chubb, J. C., Hammerschmidt, K., & Milinski, M. (2009). When should a trophically transmitted parasite manipulate its host?. *Evolution: International Journal of Organic Evolution*, 63, 448-458.

Parker, G.A., Ball, M.A., & Chubb, J.C. (2015). Evolution of complex life cycles in trophically transmitted helminths. I. Host incorporation and trophic ascent. *Journal of Evolutionary Biology,* 28, 267-291.

Pavlou, H.J., Neville, M.C., & Goodwin, S.F. (2014). Aggression: tachykinin is all the rage. *Current Biology*, 24, R243–R244.

Pinter-Wollman, N., Hubler, J., Holley, J.A., Franks, N.R., & Dornhaus, A. (2012). How is activity distributed among and within tasks in *Temnothorax ants*? *Behavioral Ecology and Sociobiology*, 66, 1407-1420.

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

Pohl, S., Witte, V., & Foitzik, S. (2011). Division of labor and slave raid initiation in slave-making ants. *Behavioral Ecology and Sociobiology,* 65, 2029-2036.

Poinar, G., & Yanoviak, S.P. (2008). *Myrmeconema neotropicum* ng n. sp. a new tetradonematid nematode parasitising South American populations of

Cephalotes atratus (Hymenoptera: Formicidae) with the discovery of an apparent parasite-induced host morph. *Systematic Parasitology,* 69, 145-153.

Poinar, G. (2012). Nematode parasites and associates of ants: past and present. *Psyche: A Journal of Entomology*, e192017, 1-13.

Poirotte, C., Kappeler, P.M., Ngoubangoye, B., Bourgeois, S., Moussodji, M., & Charpentier, M.J.E. (2016). Morbid attraction to leopard urine in *Toxoplasma*-infected chimpanzees. *Current Biology*, 26, R98-R99.

Ponton, F., Lalubin, F., Fromont, C., Wilson, K., Behm, C., & Simpson, S.J. (2011). Hosts use altered macronutrient intake to circumvent parasite-induced reduction in fecundity. *International Journal for Parasitology*, 41, 43-50.

Poulin, R. (1994). Meta-analysis of parasite-induced behavioural changes. *Animal Behaviour*, 48, 137–146.

Poulin, R., Brodeur, J., & Moore, J. (1994). Parasite manipulation of host behaviour: should hosts always lose? *Oikos*, 479-484.

Poulin, R. (1995). 'Adaptive' change in the behaviour of parasitized animals: a critical review. *International Journal for Parasitology*, 25, 1371–1383.

Poulin, R., & Thomas, F. (1999). Phenotypic variability induced by parasites: extent and evolutionary implications. *Parasitology Today*, 15, 28–32.

Poulin R (2000) Manipulation of host behaviour by parasites: a weakening paradigm? *Proceedings of the Royal Society of London B: Biological Sciences*, 267, 787–792.

Poulin, R., & Morand, S. (2000). The diversity of parasites. *The Quarterly Review of Biology,* 75, 277-293.

Poulin, R., Fredensborg, B.L., Hansen, E., & Leung, T.L. (2005). The true cost of host manipulation by parasites. *Behavioural Processes*, 68, 241-244.

Poulin, R. (2010). Parasite manipulation of host behaviour: an update and frequently asked questions. *Advances in The Study of Behavior*, 41, 151–186.

Poulin, R. (2014). Parasite biodiversity revisited: frontiers and constraints. *International Journal for Parasitology,* 44, 581-589.

Poulin, R., & Maure, F. (2015). Host manipulation by parasites: a look back before moving forward. *Trends in Parasitology*, 31, 563-570.

Poulin, R., & Randhawa, H.S. (2015). Evolution of parasitism along convergent lines: from ecology to genomics. *Parasitology,* 142, S6-S15.

Quevillon, L.E., & Hughes, D.P. (2018). Pathogens, parasites, and parasitoids of ants: a synthesis of parasite biodiversity and epidemiological traits. *bioRxiv*, 384495.

R Core Team. (2012). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Ratnieks, F.L., & Wenseleers, T. (2005). Policing insect societies. *Science*, 307, 54-56.

Rauch, G., Kalbe, M., & Reusch, T.B.H. (2005). How a complex life cycle can improve a parasite's sex life. *Journal of Evolutionary Biology,* 18, 1069-1075.

Ravary, F., Lecoutey, E., Kaminski, G., Châline, N., & Jaisson, P. (2007). Individual experience alone can generate lasting division of labor in ants. *Current Biology*, 17, 1308-1312.

Reeve, H.K. (1989). The evolution of conspecific acceptance thresholds. *The American Naturalist*, 133, 407–435.

Reilly, D.R.O., Brown, M.R., & Miller, L.K. (1992). Alteration of ecdysteroid metabolism due to baculovirus infection of the fall armyworm *Spodoptera frugiperda*: host ecdysteroids are conjugated with galactose. *Insect Biochemistry and Molecular Biology*, 22, 313–320.

Richard, F.J., Aubert, A., & Grozinger, C.M. (2008). Modulation of social interactions by immune stimulation in honey bee, *Apis mellifera*, workers. *BMC Biology*, 6, 50.

Robinson, G.E. (1992). Regulation of division of labor in insect societies. *Annual Review of Entomology*, 37, 637-665.

Robinson, E.J.H., Feinerman, O., & Franks, N.R. (2009). Flexible task allocation and the organization of work in ants. *Proceedings of the Royal Society of London B: Biological Sciences*, rspb20091244.

Robinson, E. J., Feinerman, O., & Franks, N. R. (2012). Experience, corpulence and decision making in ant foraging. *Journal of Experimental Biology*, 215, 2653-2659.

Robinson, M.D., McCarthy, D.J., & Smyth, G.K. (2010). edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics*, 26, 139–140.

Rogers, M.E., & Bates, P.A. (2007). *Leishmania* manipulation of sand fly feeding behavior results in enhanced transmission. *PLoS Pathogens*, 3, e91.

Rosenberg, L.A., Pflüger, H.J., Wegener, G., & Libersat, F. (2006). Wasp venom injected into the prey's brain modulates thoracic identified monoaminergic neurons. *Journal of Neurobiology*, 66, 155-168.

Roulston, T.H., Buczkowski, G., & Silverman, J. (2003). Nestmate discrimination in ants: effect of bioassay on aggressive behavior. *Insectes Sociaux*, 50, 151–159.

Rueppell, O., Hayworth, M.K., & Ross, N.P. (2010). Altruistic self-removal of health-comprised honey bee workers from their hive. *Journal of Evolutionary Biology*, 23, 1538–1546.

Rzezniczak, T. Z., Douglas, L. A., Watterson, J. H., & Merritt, T. J. S. (2011). Paraquat administration in *Drosophila* for use in metabolic studies of oxidative stress. *Analytical Biochemistry*, 419, 345-347.

Salvy, M., Martin, C., Bagnères, A.G., Provost, E., Roux, M., Le Conte, Y., & Clement, J.L. (2001). Modifications of the cuticular hydrocarbon profile of *Apis mellifera* worker bees in the presence of the ectoparasitic mite *Varroa jacobsoni* in brood cells. *Parasitology,* 122, 145–159.

Scharf, I., Pamminger, T., & Foitzik, S. (2011). Differential response of ant colonies to intruders: attack strategies correlate with potential threat. *Ethology*, 117, 731–739.

Scharf, I., Modlmeier, A.P., Fries, S., Tirard, C., & Foitzik, S. (2012a). Characterizing the collective personality of ant societies: aggressive colonies do not abandon their home. *PLoS One*, 7, e33314.

Scharf, I., Modlmeier, A.P., Beros, S., & Foitzik, S. (2012b). Ant societies buffer individual-level effects of parasite infections. *The American Naturalist*, 180, 671–683.

Scharsack, J.P., Koch, K., & Hammerschmidt, K. (2007). Who is in control of the stickleback immune system: interactions between *Schistocephalus solidus* and its specific vertebrate host. *Proceedings of the Royal Society of London B: Biological Sciences*, 274, 3151-3158.

Schmid-Hempel, P. (1998). Parasites in social insects. *Princeton University Press*, Princeton, NJ.

Schmid-Hempel, P. (2011). Evolutionary Parasitology. The integrated study of infections, immunology, ecology, and genetics. *Oxford University Press*, Oxford, UK.

Schneider, S.A., Scharffetter, C., Wagner, A.E., Boesch, C., Bruchhaus, I., Rimbach, G., & Roeder, T. (2016). Social stress increases the susceptibility to infection in the ant *Harpegnathos saltator. Scientific Reports,* 6, 25800.

Seehuus, S. C., Norberg, K., Gimsa, U., Krekling, T., & Amdam, G. V. (2006). Reproductive protein protects functionally sterile honey bee workers from oxidative stress. *Proceedings of the National Academy of Sciences*, 103, 962-967.

Sendova-Franks, A.B., & Franks, N.R. (1995). Spatial relationships within nests of the ant *Leptothorax unifasciatus* (Latr.) and their implications for the division of labour. *Animal Behaviour*, 50, 121-136.

Seppälä, O., Karvonen, A., & Valtonen, E.T. (2004). Parasite-induced change in host behaviour and susceptibility to predation in an eye fluke–fish interaction. *Animal Behaviour*, 68, 257-263.

Seppälä, O., Karvonen, A., & Valtonen, E.T. (2005). Manipulation of fish host by eye flukes in relation to cataract formation and parasite infectivity. *Animal Behaviour*, 70, 889-894.

Seppälä, O., & Jokela, J. (2008). Host manipulation as a parasite transmission strategy when manipulation is exploited by non-host predators. *Biology Letters,* 4, 663-666.

Seppälä, O., Valtonen, E.T., & Benesh, D.P. (2008). Host manipulation by parasites in the world of dead-end predators: adaptation to enhance transmission? *Proceedings of the Royal Society of London B: Biological Sciences*, 275, 1611-1615.

Shykoff, J.A., & Schmid-Hempel, P. (1991). Parasites delay worker reproduction in bumblebees: consequences for eusociality. *Behavioral Ecology*, 2, 242-248.

Smallegange, R.C., van Gemert, G.J., van de Vegte-Bolmer, M., Gezan, S., Takken, W., Sauerwein, R.W., & Logan, J.G. (2013). Malaria infected mosquitoes express enhanced attraction to human odor. *PloS One,* 8, e63602.

Smith, C. R., Suarez, A. V., Tsutsui, N. D., Wittman, S. E., Edmonds, B., Freauff, A., & Tillberg, C. V. (2011). Nutritional asymmetries are related to division of labor in a queenless ant. *PLoS One*, 6, e24011.

Sohal, R.S., Toroser, D., & Bre, C. (2008). Age-related decrease in expression of mitochondrial DNA encoded subunits of cytochrome c oxidase in *Drosophila melanogaster*. *Mechanisms of Ageing and Development*, 129, 558–561.

Soroker, V., Vienne, C., & Hefetz, A. (1995). Hydrocarbon dynamics within and between nestmates in *Cataglyphis niger* (Hymenoptera: Formicidae). *Journal of Chemical Ecology*, 21, 365–378.

Starks, P.T., Blackie, C.A., & Seeley, T.D. (2000). Fever in honeybee colonies. *Naturwissenschaften*, 87, 229-231.

Stearns, S. C. (1992). The evolution of life histories. *Oxford Univeristy Press*. Oxford, UK.

Stoldt, M., Beros, S., Butter, F., & Foitzik, S. Molecular cross-talk between a social species and its tapeworm parasite: a proteomics approach. In preparation.

Stroeymeyt, N., Brunner, E., & Heinze, J. (2007). 'Selfish worker policing' controls reproduction in a *Temnothorax* ant. *Behavioral Ecology and Sociobiology*, 61, 1449–1457.

Stroeymeyt, N., Casillas-Pérez, B., & Cremer, S. (2014). Organisational immunity in social insects. *Current Opinion in Insect Science*, 5, 1-15.

Stroeymeyt, N., Grasse, A. V., Crespi, A., Mersch, D. P., Cremer, S., & Keller, L. (2018). Social network plasticity decreases disease transmission in a eusocial insect. *Science*, 362, 941-945.

Stroeymeyt, N., Joye, P., & Keller, L. (2017). Polydomy enhances foraging performance in ant colonies. *Proceedings of the Royal Society of London B: Biological Sciences*, 284, 20170269.

Stuart, R.J. (1988). Collective cues as a basis for nestmate recognition in polygynous leptothoracine ants. *Proceedings of the National Academy of Sciences of the USA*, 85, 4572–4575.

Sturgis, S.J., & Gordon, D.M. (2012). Aggression is task dependent in the red harvester ant (*Pogonomyrmex barbatus*). *Behavior Ecology*, 24, 532– 539.

Szathmáry, E., & Smith, J.M. (1995). The major evolutionary transitions. *Nature*, 374, 227-232.

Tanner, C.J., & Adler, F.R. (2009). To fight or not to fight: context-dependent interspecific aggression in competing ants. *Animal Behaviour*, 77, 297– 305.

Tasaki, E., Kobayashi, K., Matsuura, K., & Iuchi, Y. (2018). Long-lived termite queens exhibit high Cu/Zn-superoxide dismutase activity. *Oxidative Medicine and Cellular Longevity*, 2018, 8.

Terrapon, N., Li, C., Robertson, H.M., et al. (2014). Molecular traces of alternative social organization in a termite genome. *Nature Communications*, 5, 3636.

Thomas, J.A., & Elmes, G.W. (1998). Higher productivity at the cost of increased host-specificity when *Maculinea* butterfly larvae exploit ant colonies through trophallaxis rather than by predation. *Ecological Entomology*, 23, 457-464.

Thomas, F., & Poulin, R. (1998). Manipulation of a mollusc by a trophically transmitted parasite: convergent evolution or phylogenetic inheritance? *Parasitology*, 116, 431-436.

Thomas, F., Schmidt-Rhaesa, A., Martin, G., Manu, C., Durand, P., & Renaud, F. (2002). Do hairworms (Nematomorpha) manipulate the water seeking behaviour of their terrestrial hosts? *Journal of Evolutionary Biology*, 15, 356–361.

Thomas, F., Adamo, S., & Moore, J. (2005). Parasitic manipulation: where are we and where should we go? *Behavioural Processess,* 68, 185–199.

Thomas, F., Poulin, R., & Brodeur, J. (2010). Host manipulation by parasites: a multidimensional phenomenon. *Oikos*, 119, 1217–1223.

Thompson, G.J., Crozier, Y.C., & Crozier, R.H. (2003). Isolation and characterization of a termite transferrin gene up-regulated on infection. *Insect Molecular Biology*, 12, 1-7.

Tofilski, A. (2009). Shorter-lived workers start foraging earlier. *Insectes Sociaux*, 56, 359-366.

Toth, A. L., Kantarovich, S., Meisel, A. F., & Robinson, G. E. (2005). Nutritional status influences socially regulated foraging ontogeny in honey bees. *Journal of Experimental Biology*, 208, 4641-4649.

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 endoparasite *Anomotaenia brevis*. *Journal of Insect Physiology*, 46, 169–178.

Trapnell, C., Pachter, L., & Salzberg, S.L. (2009). TopHat: discovering splice junctions with RNA-Seq. *Bioinformatics*, 25, 1105–1111.

True, J.R. (2003) Insect melanism: the molecules matter. *Trends in Ecology and Evolution*, 18, 640–647.

Tsuji, K., Kikuta, N., & Kikuchi, T. (2012). Determination of the cost of worker reproduction via diminished lifespan in the ant *Diacamma* sp. *Evolution*, 66, 1322-1331.

Ugelvig, L.V., & Cremer, S. (2007). Social prophylaxis: group interaction promotes collective immunity in ant colonies. *Current Biology*, 17, 1967-1971.

Valles, S.M., & Pereira, R.M. (2005). *Solenopsis invicta* transferrin: cDNA cloning, gene architecture, and up-regulation in response to *Beauveria bassiana* infection. *Gene*, 358, 60-66.

Van Houte, S., Ros, V.I.D., & Van Oers, M.M. (2013). Walking with insects: molecular mechanisms behind parasitic manipulation of host behaviour. *Molecular Ecology*, 22, 3458–3475.

Van Houte, S., van Oers, M.M., Han, Y., Vlak, J.M., & Ros, V.I. (2014). Baculovirus infection triggers a positive phototactic response in caterpillars to induce 'tree-top' disease. *Biology Letters*, 10, 20140680.

Van Wilgenburg, E., Felden, A., Choe, D.H., Sulc, R., Luo, J., Shea, K.J., Elgar, M.A., & Tsutsui, N.D. (2011). Learning and discrimination of cuticular hydrocarbons in a social insect. *Biology Letters*, rsbl20110643.

Van Zweden, J.S., & D'Ettorre, P. (2010). Nestmate recognition in social insects and the role of hydrocarbons. In G.J. Blomquist & A.G. Bagnères (Eds). *Insect hydrocarbons: biology, biochemistry and chemical ecology* (pp. 222–243).

Venables, W.N., & Ripley, B.D. (2002). Modern applied statistics with S, *Springer*, New York, NY.

Verble, R.M., Meyer, A.D., Kleve, M.G., & Yanoviak, S.P. (2012). Exoskeletal thinning in *Cephalotes atratus* ants (Hymenoptera: Formicidae) parasitized by *Myrmeconema neotropicum* (Nematoda: Tetradonematidae). *Journal of Parasitology,* 98, 226-228.

Vézilier, J., Nicot, A., Gandon, S., & Rivero, A. (2012). *Plasmodium* infection decreases fecundity and increases survival of mosquitoes. *Proceedings of the Royal Society of London B: Biological Sciences*, 279, 4033-4041.

Vienne, N.C., Soroker, V., & Hefetz, A. (1995). Congruency of hydrocarbon patterns in heterospecific groups of ants: transfer and/or biosynthesis? *Insectes Sociaux*, 42, 267–277.

Von Wyschetzki, K., Rueppell, O., Oettler, J., & Heinze, J. (2015). Transcriptomic signatures mirror the lack of the fecundity/longevity trade-off in ant queens. *Molecular Biology and Evolution*, 32, 3173–3185.

Vyas, A., Kim, S.K., & Sapolsky, R.M. (2007). The effects of toxoplasma infection on rodent behavior are dependent on dose of the stimulus. *Neuroscience*, 148, 342-348.

Waddington, K.D., & Rothenbuhler, W.C. (1976). Behaviour associated with hairless-black syndrome of adult honeybees. *Journal of Apicultural Research,* 15, 35-41.

Waddington, S.J., & Hughes, W.O. (2010). Waste management in the leaf-cutting ant *Acromyrmex echinatior*: the role of worker size, age and plasticity. *Behavioral Ecology and Sociobiology*, 64, 1219-1228.

Wagner, D., Brown, M.J., Broun, P., Cuevas, W., Moses, L.E., Chao, D.L., & Gordon, D.M. (1998). Task-related differences in the cuticular hydrocarbon composition of harvester ants. *Pogonomyrmex barbatus*. *Journal of Chemical Ecology*, 24, 2021–2037.

Wagner, D., Tissot, M., Cuevas, W., & Gordon, D.M. (2000). Harvester ants utilize cuticular hydrocarbons in nestmate recognition. *Journal of Chemical Ecology*, 26, 2245–2257.

Wakonigg, G., Eveleigh, L., Arnold, G., & Crailsheim, K. (2000). Cuticular hydrocarbon profiles reveal age-related changes in honey bee drones (*Apis mellifera carnica*). *Journal of Apicultural Research*, 39, 137–141.

Walker, T.N., & Hughes, W.H.O. (2009). Adaptive social immunity in leafcutting ants. *Biology Letters*, 5, 446–448.

Warr, E., Meredith, J.M., Nimmo, D.D., Basu, S., Hurd, H., & Eggleston, P. (2006). A tapeworm molecule manipulates vitellogenin expression in the beetle *Tenebrio molitor*. *Insect Molecular Biology*, 15, 497-505.

Weatherly, N. F. (1970). Increased survival of Swiss mice given sublethal infections of *Trichinella spiralis*. *The Journal of Parasitology*, 56, 748-752.

Webb, T.J., & Hurd, H. (1999). Direct manipulation of insect reproduction by agents of parasite origin. *Proceedings of the Royal Society of London B: Biological Sciences*, 266, 1537-1541.

Weiner, S. A., Geffre, A. G., & Toth, A. L. (2017). Functional genomics in the wild: a case study with paper wasps shows challenges and prospects for RNA interference in ecological systems. *Genome*, 61, 266-272.

Weinreich, F., Benesh, D.P., & Milinski, M. (2013). Suppression of predation on the intermediate host by two trophically-transmitted parasites when uninfective. *Parasitology*, 140, 129-135.

Weinstein, S.B., & Kuris, A.M. (2016). Independent origins of parasitism in Animalia. *Biology Letters*, 12, 20160324.

Wesołowska, W., & Wesołowski, T. (2014). Do *Leucochloridium* sporocysts manipulate the behaviour of their snail hosts? *Journal of Zoology*, 292, 151-155.

Wilson, E.O. (1971). The insect societies. *Harvard University Press*, Cambridge, MA, USA.

Wilson, E.O. (1987). Causes of ecological success: the case of the ants. *Journal of Animal Ecology*, 56, 1-9.

Wilson, E.O., & Hölldobler, B. (2005). Eusociality: origin and consequences. *Proceedings of the National Academy of Sciences*, 102, 13367-13371.

Worden, B.D., Parker, P.G., & Pappas, P.W. (2000). Parasites reduce attractiveness and reproductive success in male grain beetles. *Animal Behaviour*, 59, 543-550.

Wurm, Y., Wang, J., Riba-Grognuz, O., et al. (2010). The genome of the fire ant *Solenopsis invicta*. *Proceedings of the National Academy of Sciences of the USA*, 108, 5679–5684.

Xue, S., & Barna, M. (2012). Specialized ribosomes: a new frontier in gene regulation and organismal biology. *Nature Reviews Molecular Cell Biology*, 13, 355–369.

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

Yoshiga, T., Hernandez, V. P., Fallon, A. M., & Law, J. H. (1997). Mosquito transferrin, an acute-phase protein that is up-regulated upon infection. *Proceedings of the National Academy of Sciences*, 94, 12337-12342.

Zelmer, D.A. (1998). An evolutionary definition of parasitism. *International Journal for Parasitology*, 28, 531.

Zhang, B., & Horvath, S. (2005). A general framework for weighted gene co-expression network analysis. *Statistical Applications in Genetics and Molecular Biology*, 4, 17.

Zhukovskaya, M., Yanagawa, A., & Forschler, B.T. (2013). Grooming behavior as a mechanism of insect disease defense. *Insects,* 4, 609-630.

Acknowledgments

To advisors, colleagues, friends and family

[removed for privacy purposes]

Curriculum Vitae

Sara Beros

[removed for privacy purposes]