Individual stress-resistance in the ant *Formica exsecta*

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Academic Dissertation

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The different accidents of life are not so changeable as the feelings of human nature. I had worked hard for [ ] years, for the sole purpose of infusing life into an inanimate body. For this I had deprived myself of rest and health. I had desired it with an ardour that far exceeded moderation; but now that I had finished, the beauty of the dream vanished, and breathless horror and disgust filled my heart.
– *Victor Frankenstein*
(from Frankenstein*; or, The Modern Prometheus* by M. Shelley)

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Stress responses upon starvation and exposure to bacteria in the ant *Formica exsecta*
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DS conducted the experimental work with the help of DF and NB. DS performed the laboratory work (qPCR), and statistical analysis of the survival and qPCR data. The study is based on an original idea by DS and LS, later modified by all authors. All authors also contributed to the study design and manuscript preparation.

Chapter two

Inbreeding-related trade-offs in stress resistance in the ant *Formica exsecta*
D. Freitak, N. Bos, D. Stucki, and L. Sundström, 2014, Biology Letters 10: 20140805. http://dx.doi.org/10.1098/rsbl.2014.0805

DF, NB and DS conducted the experimental work. DF performed the laboratory work (qPCR). DS and NB did the statistical analysis of the survival data, and NB did the statistical analysis of the qPCR data. The study is based on an original idea by LS, later modified by all authors. All authors also contributed to the study design and manuscript preparation.

Chapter three

Caste-specific expression of constitutive and *Beauveria bassiana* induced immunity in the ant *Formica exsecta*
D. Stucki, L. Sundström, and D. Freitak, Accepted for publication in Myrmecological News.

DS conducted the experimental work. DS performed the laboratory work (qPCR), and statistical analysis of the survival and qPCR data. The study is based on an original idea by LS and DF, later modified by all authors. All authors also contributed to the study design and manuscript preparation.

Chapter four

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D. Stucki, D. Freitak, and L. Sundström, Accepted for publication in PLOS One.

DS conducted the experimental work. DS performed the laboratory work (qPCR), and statistical analysis of the survival and qPCR data. The study is based on an original idea by DS, later modified by all authors. All authors also contributed to the study design and manuscript preparation.

Abstract

Compared to solitary insects, the social organization and interactions in eusocial insects are expected to render them more vulnerable to parasitic exploitation. As a consequence, eusocial insects in turn evolved social immune defenses – sophisticated behaviors that complement the individual immune defenses and increase their resistance against parasitic exploitation. Thus, within the network of the eusocial community, host-parasite interactions occur not only between a single host and its parasites, but among all individuals in the network. Yet, although in eusocial insects host-parasite interactions and other ecological stresses affect the entire community, the individuals provide the basic physiological responses in the defense against external influences. Thus, individual stress responses are an important factor in mediating the variation within the colony to ecological stresses. In this thesis I investigated the physiological stress responses of the ant *F. exsecta* in the light of host-parasite interactions. I found that oral exposure to bacteria can have a beneficial effect on the survival of food deprivation. Yet, the response to the infection in combination with starvation showed temporal variation. Furthermore, I found striking similarities in the immune responses of young males and foraging workers, as compared to young queens and nursing workers. This suggests that the residual life expectancy may have a role in mediating immune defenses among the castes of *F. exsecta*. Stress responses not only varied among the castes, but also between two natural populations. I found different reaction norms to temperature and humidity, possibly due to the different environmental conditions that the ants experience in their natural habitat. Given that these reaction norms also affected the expression of immune genes, it is likely that variation in the environmental conditions can affect the immune defenses, and thus, may influence host-parasite interactions. In conclusion, this thesis provides insight into the impact of ecological factors on the resistance and responses to stress in a social insect. I show that individual stress responses are tightly linked to immune defenses, which in turn may affect the stress response of the entire community. As a consequence, variation in the environmental conditions, and thus, exposure to different ecological stresses, may result in different evolutionary trajectories among populations, and even among colonies.

Background

One of the major characteristics that distinguishes eusocial insects from solitary insects, is their ability to allocate different tasks to separate castes – specialized individuals which are phenotypically and behaviorally adapted to fulfill a particular task (Oster & Wilson 1979; E. O. Wilson 1971). While the reproductive castes (queens and males) play the central role in reproduction, depending on the species, one or several worker castes perform the maintenance tasks for the entire colony. These tasks adopted by the workers involve foraging, processing resources, taking care of the brood, or cleaning and defending the colony. Mostly these worker castes are sterile or suppressed from reproduction. Yet, despite the lack of direct reproductive fitness in workers, and therefore, the absence of direct inheritance of the social traits, eusocial insects evolved to become a group of the most successful organisms in almost all biomes.

However, compared to solitary insects, eusocial insects feature traits that – in theory – render them more vulnerable to parasitic exploitation. For example the close relatedness and the high density within a colony of eusocial insects likely facilitate the horizontal transfer of pathogens. The resulting increase in selection pressure from parasites led to the evolution of a variety of unique traits in eusocial insects, such as social immune defenses (Cremer et al. 2007). Such social immunity provides an additional layer of immune defenses through behaviors targeted towards nest-mates or the nest as such. One particularly sophisticated behavior is the use of the antimicrobial properties of resin. Various social insects increase nest hygiene by collecting resin from the surrounding environment to disinfect the nest (Chapuisat et al. 2007; Leonhardt & Blüthgen 2009; Simone et al. 2009). Similarly, many species remove corpses from the colony to prevent epidemics (Howard & Tschinkel 1976; Trumbo et al. 1997). In addition, almost all social insects remove fungal spores not only from themselves, but also from nest-mates through allogrooming (Hughes et al. 2002; Oi & Pereira 1993; Wilson-Rich et al. 2007). A few eusocial insects were even shown to benefit from increased immune defenses by vaccination. In some

ants and termites exposure of naïve individuals to previously immune-challenged individuals increased the survival of the naïve individuals upon pathogen infection (Hamilton et al. 2011; Traniello et al. 2002; Ugelvig & Cremer 2007). This effect is possibly achieved through the trophallactic exchange of immune-priming components, such as degraded bacterial cell walls (Hamilton et al. 2011).

As a consequence, within the network of a eusocial community, host-parasite interactions are not only occurring between a single host and its parasites, but among all individuals in the network. Therefore, in eusocial insects host-parasite interactions do not only affect the life history of single individuals but of the entire colony. For example, one of the most dominant traits of eusocial insects, the reproductive division of labor, was suggested to have evolved due to the increased parasite pressure in socially living groups (O'Donnell 1997). Similarly, the evolution of different mating types in eusocial insects (polyandry/polygyny) is presumably associated with evolutionary dynamics of host-parasite interactions (Sherman et al. 1988). Vice versa, the social features of eusocial insects can have an impact on various life history dependent host-parasite interactions. In many eusocial insects, the workers perform their tasks sequentially during their life (Oster & Wilson 1979; E. O. Wilson 1971). In these communities, younger individuals remain close to the nest and perform brood care and nest cleaning, whereas older individuals pursue more risky tasks such as foraging and nest defense. Such differences in both age and task, may create differences in the immune defenses of these individuals. Similarly, trade-offs may be altered or masked by social features. For example, the exchange of nutrition among individuals could have an influence on the trade-off between the physiological condition and the immune defenses of an individual. Given that immune defenses are energetically costly to mount (McKean et al. 2008; Siva-Jothy & Thompson 2002), trade-offs are likely to occur when individuals are starved. Yet, frequent social interactions among nest-mates can increase starvation-resistance and therefore reduce the impact of nutrition-dependent trade-offs (Rueppell & Kirkman 2005).

In conclusion, immune defenses, life histories, and life history trade-offs are tightly linked to the sociality of these insect communities. Thus, the social features of eusocial insects likely cause variation in the expression of trade-offs that involve individual immune defenses. This differential expression may cause variation in the selection pressure from different stresses, and thus can have an impact on the evolution of a species, provided there is variation in the expression of the trade-offs.

Individual insect immune defenses

Insects rely on a whole palette of defense mechanisms to defend themselves against the vast diversity of exploiting parasites and pathogens. Already before an infection, behavioral and physical barriers provide protection against the constant challenge by potential parasites. Behaviorally, insects can detect and avoid possible sources of infection (Meyling & Pell 2006; Parker et al. 2010) or groom themselves regularly to reduce the risk of an infection (Valentine 1973). If a parasite comes in contact with an insect, the thick insect cuticle constitutes a hardly penetrable physical barrier. However, the solid cuticle is not only a physical barrier, it is furthermore covered with different chemical compounds, lethal to microbes and other organisms that try to infect the insects through the cuticle (Ashida & Brey 1995; Brey et al. 1993). Similarly. the digestive system of insects contains a cocktail of peptides and chemicals directed against any undesired invader. A major part of these compounds are antimicrobial peptides, short proteins that recognize and/or attack possible pathogens. Upon recognition of a potential threat in the gut, the amount of antimicrobial peptides and other defense components is even increased (Hao et al. 2003; Lehane et al. 1997; K. Wilson et al. 2001).

Once a pathogen successfully penetrates the host's cuticle, insects can activate several defense mechanisms in order to remove the threat from the system. The Toll pathway – named after the transmembrane receptor Toll – predominantly responds to the recognition of Gram positive bacteria and

fungal pathogens via the respective recognition molecules (Lemaitre et al. 1996). For example, the fungal cell wall component β-1,3-glucan is recognized by a β-1,3-glucan-binding protein (β1,3g), which leads to the activation of the Toll pathway (Brown & Gordon 2005). Upon activation, the Toll pathway triggers the expression of antimicrobial peptides, specifically targeted against fungi and Gram positive bacteria, such as *defensin* or *dorsomycin* (Anderson 2000). In addition to the activation of the Toll pathway, β1,3g also activates the proPhenoloxidase (PPO) pathway (Brown & Gordon 2005; Söderhäll & Cerenius 1998). The PPO pathway is directed against all forms of parasites, and can also be activated through recognition proteins that bind to the cellular membrane component lipopolysaccharide of Gram negative bacteria (Söderhäll & Cerenius 1998). The PPO pathway results in the cleavage of the the inactive precursor protein proPhenoloxidase into Phenoloxidase, which directs the accumulation of melanin at the site of the disturbance, and isolates the parasite from the hemolymph by encapsulation. This process renders the parasite immobile and leaves it to starvation or asphyxia (Söderhäll & Smith 1986). The PPO pathway is further involved in the response to physical damage of the insect cuticle and consequent wound healing (Theopold et al. 2002).

The Imd pathway constitutes another important immune pathway in insects. This pathway is activated upon recognition of Gram negative bacteria, via the respective recognition proteins, and results in the expression of antimicrobial peptides such as hymenoptaecins or cecropins (Myllymäki et al. 2014; Tingvall et al. 2001). The Imd pathway also regulates the expression of the transcription factor NfkB or the enzyme dual oxidase, which mediates the immune defense via reactive oxygen species (ROS) (Ryu et al. 2006). Oxygen radicals can cause severe cell damage due to their ability to interfere with most biologically active macromolecules. In low doses, and concentrated at the location of a potential infection, the cytotoxic reactive oxygen species can be targeted against many types of invading parasites. Yet, as the radicals also inflict

damage to the host's system, production and local application of ROS is tightly regulated (Molina-Cruz et al. 2008; Nappi et al. 2000).

In addition to the expression of genes that are directly involved in the immune defense, infections also result in the regulation of other functions, such as the mobilization of resources and the protection from autoimmunity. As reactive oxygen species are highly cytotoxic, antioxidant enzymes, as for example peroxidase or superoxide dismutases, are expressed, in order to limit self-damage from ROS (Hsu & Hsieh 2014; Kumar et al. 2010). Furthermore, given that immune defenses are often energetically costly, metabolic pathways, such as the insulin-like-protein pathway (DiAngelo et al. 2009), and storage proteins such as vitellogenins and arylphorins, which also carry some pathogen-recognition-segments (Amdam et al. 2004; Zhu et al. 2009), are often associated with immune defenses.

Aims of the Thesis

In this thesis I aimed to investigate stress resistance in the light of host-parasite interactions in the eusocial ant *Formica exsecta*. I investigated how various stresses influence the expression of immune defenses and how stress-resistance is traded off with immune defenses in the ant *F. exsecta*. Chapter one aimed to reveal the underlying mechanisms of a trade-off between starvation-resistance and immune defenses. With the analysis of gene expression in starved and infected ants I intended to show how the expression of particular genes is associated with the survival upon exposure to two stresses, starvation and infection. In Chapter two, I investigated a potential influence of inbreeding depression on the resistance of the ants to oxidative stress, and how inbreeding affects the expression of stress-resistance genes. In Chapter three, I investigated the variation in immune defenses among the different castes of *F. exsecta*. The Chapter aimed to reveal how the different castes and worker classes – queens, males, nurses, and foragers – invest into constitutive and induced immune defenses, according to their life histories. Chapter four aimed to investigate

the expression of genes related to stress-resistance, including immune defenses, among two populations of *F. exsecta*. In this project I tested how environmental conditions, in particular temperature and humidity, affect the expression of genes associated to stress resistance and immune defenses. Furthermore, I tested for potential differences in the response to these environmental conditions between two natural populations. In combination these thesis chapters provide insight into the individual stress resistances in a social insect.

Materials and Methods

Study system

Formica exsecta

The excised wood ant *Formica exsecta* Nyl. is a palaearctic ant species distributed throughout Europe and Asia (Czechowski et al. 2002; Goropashnaya et al. 2007). This ecotone, mound-building species nests predominantly near forest edges and in open patches in mixed and deciduous forests. Colony foundation can occur either independently, socio-parasitic on nests of other wood ants, or socially by joining an established colony. Due to high aggression from monogyne colonies, social conjunction requires a queenless nest, or an already established polygynous colony. In northern Europe, new alates (queens/males) usually emerge in July, however, whether, and which alates are produced can vary between years and colonies (Liautard et al. 2003). During the mating flight between July and August, both alates mate multiple times, after which the males die within days, whereas, queens may maintain offspring production for several decades (Pamilo 1991). Workers are produced in two broadly defined cohorts, one of which develops from eggs laid in May-June and ecloses in July, and another which develops from eggs laid in July-August and ecloses in late August-early September. The worker caste of *F. exsecta* is polyethic, that is, the workers assume different tasks throughout their life. The two behavioral castes, nurses and foragers, broadly coincide with the production of the two cohorts. When queens resume egg production after

hibernation, the cohort born first in the previous year starts foraging, whereas the second cohort from the previous year remains inside the nest and rears the first cohort of the current year. This new cohort assumes indoor tasks, and raises the second cohort. The overwintered workers gradually pass away in August and September, although a small fraction may survive a second hibernation. Hibernation normally starts by the end of September.

For the experiments I collected *F. exsecta* ants from two populations in southwestern Finland. The population at Tvärminne Zoological Station is distributed to several islands near the coast, of which I sampled from Alören (*59°50'06"N 23°15'50"E*; Chapter three), Furuskär (*59°50'00"N 23°16'05"E*; Chapters two, three, and four), Joskär (*59°50'42"N 23°15'21"E*; Chapters two and three), and Rovholmen (*59°50'15"N 23°15'08"E*; Chapter two). The Tvärminne population has been monitored since 1994 (Haag-Liautard et al. 2008). In general these islands feature bedrock and moraine soil, and the islands are partly covered by sparse pine forests. The Prästkulla population is located on the mainland, 16km (as the crow flies) from Tvärminne Zoological Station (*59°58'44.6"N 23°20'50.9"E*; Chapters one and four). The sampled nests came from a dense pine forest, which was covered by layer of moss on the ground.

Pathogens

For the bacteria infections (Chapter one), I used the Gram negative bacteria *Serratia marcescens* (DB10), *Pseudomonas entomophila* (L48), and *Escherichia coli* (K12). *S. marcescens* and *P. entomophila* are soil dwelling opportunistic entomopathogens, with a wide range of potential hosts (Grimont & Grimont 1978; Vallet-Gely et al. 2010). Both bacteria-strains were originally isolated from *D. melanogaster* and were shown to infect insects through oral ingestion (Dillon et al. 2005; Flyg et al. 1980; Vodovar et al. 2005, 2006).

For the fungus infections (Chapter three), I used the entomopathogenic fungus *Beauveria bassiana* (KVL 03-90). The fungus *B. bassiana* is

extensively used in research on ant immunity, and the infection process is well described (Gillespie et al. 2000). The spores of the fungus attach to the cuticle of an insect and form an appressorium. From the appressorim the hyphae penetrate the cuticle and spread inside the haemocoel. After the death of the individual, the fungus produces external conidiphores, which contain new spores.

Experimental procedures

Standard ant-maintenance conditions

For all experiments the ants were collected from the field in zip-bags with nest-material, and then transferred to 30cm x 20cm x 15cm nest boxes. The ants were then kept at room temperature until the setup of the experiments, with daily provisioning of *ad libitum* standard diet. During the experiments the ants were kept in plaster-lined pots (Ø: 7cm, h: 5cm), with a Fluon® coating (Whitford) and a perforated lid. The ants were fed daily with Bhatkar-Whitcomb diet based on agar, honey and egg (Bhatkar & Whitcomb 1970). To maintain humidity, an open 1.5ml tube filled with water and a piece of cotton was placed in each pot, so that the relative humidity was maintained at ca. 70%.

Infections

Infections were either done by supplementing the food with live bacteria (Chapter one) or by submerging the ants in a solution containing spores of the entomopathogenic fungus *Beauveria bassiana* (Chapter three). For the bacteria infections, a growth-culture was prepared for *S. marcescens* and renewed daily by inoculation with 30µl of overnight culture in 10ml LB-medium at 37°C without shaking. The ants were fed daily ~100µl standard diet supplemented with 50µl of concentrated bacterial growth-culture. The bacterial culture was concentrated by centrifuging the overnight culture for 3min at 8,000rpm, subsequent removal of all liquid LB-medium and suspension of the remaining pellet in 1ml clear LB-medium, which led to a final concentration of ca. 10^8 cfu/ml. The control ants received ~100µl standard diet supplemented with 50µl pure LB-medium.

For the fungus infections, spores of the entomopathogenic fungus *Beauveria bassiana* were suspended in Triton X-100 (Sigma-Aldrich) at a concentration of ~$1x10^8$ spores/ml, and the ants were submerged for five seconds in the spore-solution. The control ants were submerged for five seconds into pure Triton X-100.

Survival, RNA-sampling and sampling for lytic activity

During each experiment, the survival of the ants was recorded on a daily basis, and dead individuals were removed from the pots. For gene expression analysis, a pooled sample of multiple live ants was obtained per biological replicate. The ants were transferred to 200µl - 500µl RNA isolation reagent (RNALater, AMBION; Isol-RNA Lysis Reagent, 5 Prime; TRIsure, Bioline), cut into small pieces, and stored at -80°C until further processing. For the analysis of the antibacterial (lytic) activity of the hemolymph, for each biological replicate two ants were separated from the poison gland, and homogenized in 40µl of ice cold phosphate-buffered saline, and stored at -80°C until further processing.

Chapter-specific procedures

Chapter one

Prästkulla, 2013: From each of ten colonies, 160 ants were evenly distributed to eight experiment-pots. Each of the eight pots was then randomly assigned to one of four Exposure treatments (Control; *S. marcescens*; *E. coli*; *P. entomophila*), and one of two Starvation treatments (*ad libitum*; Starvation). For seven days, the ants in all pots were fed daily standard diet with the respective supplement. Subsequently, the ants assigned for the starvation treatment ceased to receive any food for another seven days, whereas, the ants in the *ad libitum* fed treatment continued to receive standard diet with the respective Exposure supplement for the same time. One RNA-sample for gene expression was collected from each pot (three ants pooled) after a total of nine days in the experiment, that is, two days after the application of the Starvation treatment. The experiment was repeated two months later as a second set, with a sample size of nine colonies.

Chapter two

Furuskär/Joskär/Rovholmen, 2012: For each of 21 colonies, 144 ants were distributed evenly to four experiment-pots, for each of two treatments. The ants in a control-treatment received ~200µl standard diet on a daily basis, whereas, the ants in a ROS-treatment received ~200µl standard diet supplemented with hydrogen peroxide, adjusted to a final concentration of 4%. Four days after the start of the experiment, samples for gene expression, and antibacterial activity of the hemolymph were collected from each colony and treatment. For RNA-sampling three sets of three individuals, and for lytic activity three sets of two individuals were collected randomly from the four pots of each treatment and colony.

Chapter three

Alören/Furuskär/Joskär, 2013 & 2015: In 2013 young queens and young males were collected from 21 colonies (reproductives), and in 2015 indoor workers (nurses) and outdoor workers (foragers) were collected from 20 colonies. Just after the collections, one RNA-sample was collected to measure the constitutive level of gene expression of each group (five ants pooled). Subsequently, for the reproductives 15 ants, and for the workers 30 ants were transferred to each of two experiment-pots. Each of these two experiment-pots was then randomly assigned either to the control-treatment or the infection-treatment. After application of the respective treatment, the ants fer fed daily ~200µl standard diet for ten days. Four days after the start of the experiment one RNA-sample for induced gene expression was collected from each pot (reproductives: three ants pooled; workers: five ants pooled).

Chapter four

Furuskär/Prästkulla, 2014: Workers from inside the nest were collected from twelve colonies of Furuskär and nine colonies of Prästkulla. From each colony four separate RNA-samples were collected for pre-experiment gene expression (three ants pooled). Of the remaining ants of the collections, 20 workers per colony were placed into each of four experiment-pots. Each pot was then randomly assigned to one of two temperature-treatments (20°C /

25°C), and to one of two humidity-treatments (50% / 75%). The pots were then placed into one of two incubators set to the respective temperature and 50% relative air humidity. To the pots of the 75% humidity-treatment an open 1.5ml tube filled with water and a piece of cotton was added, whereas the pots of the 50% humidity-treatment remained without adding a tube. The ants were fed daily with ~200µl standard diet for ten days. On day ten after the onset of the laboratory treatments, I sampled three biological replicates from each pot for gene expression (three ants pooled).

Gene expression analysis

To extract the RNA of the collected ants, the thawed samples were homogenized in 600µl RNA isolation reagent (Isol-RNA Lysis Reagent, 5 Prime; TRIsure, Bioline) with two stainless steel beads using a TissueLyser (Qiagen). Subsequently, an additional 400µl RNA isolation reagent and 150µl chloroform (Sigma) or 1-bromo-3-chloropentane (Sigma) was added to the homogenized samples. After mixing, the samples were centrifuged for 10min at 13,000rpm at 4°C. Of the resulting separate phases, the upper phase, containing the RNA, was then transferred to a new, autoclaved 1.5ml tube and supplemented with 500µl isopropanol (Sigma, min. 99%). After mixing, the suspended RNA was allowed to precipitate over night at -20°C, and then the samples were centrifuged for 30min at 13,000rpm at 4°C to sediment the RNA. After removal of the supernatant, the pellet was washed on ice with 500µl 80% EtOH (Altia Oyj) and centrifuged for 10min at 13,000rpm at 4°C. The pellet was then dried at room-temperature and dissolved in autoclaved ddH_2O or RNA storage solution (AMBION). The concentration and quality of the RNA was measured photospectrometrically with NanoDrop (PEQ-Lab) and possible gDNA contamination was eliminated by DNase digest (DNase I, RNase-free; ThermoScientific; TURBO DNase, AMBION). Subsequently, cDNA was synthesized from 1µg RNA for each sample, with a blend of oligo(dT) and random primers (iScript cDNA Synthesis Kit; Bio-Rad). The cDNA was afterwards diluted in 80µl autoclaved ddH_2O for a final volume of 100µl.

Primer design for the target genes was done with the online Primer3 internet-based interface (http://www.ncbi.nlm.nih.gov/tools/primer-blast/) (Untergasser et al. 2012). All primers were designed by the rules of highest maximum efficiency and sensitivity to avoid formation of self- and hetero-dimers, hairpins and self-complementarity. Gene-specific primers were designed on the basis of the sequences obtained from *F. exsecta* transcriptome (Johansson et al. 2013). Quantitative real time PCR (qPCR) was performed on 384-well plates on a CFX384 Touch™ Real Time PCR Detection System (Biorad) using iQ™ SYBR® Green Supermix (Bio-Rad), with an initiation of 3min at 95°C, 40 cycles of 15sec at 95°C for denaturation and 45sec at 58°C for annealing/extension, and a final step for 7min at 95°C. All qPCR assays were run with two technical replicates, which were assessed for consistency and possible outliers, and subsequently averaged before normalization. Non-detects (no amplification signal within 40 cycles) were set to the maximal cycle number (i.e. Ct = 40), or removed in case the second technical replicate showed amplification.

Antibacterial activity

The plates for the lytic assay were prepared with 10ml of 1x Phosphate buffer containing 1% agar and 0,1mg of *Micrococcus lyzodeicticus* lyophilisized cells (Sigma) per 1ml of buffer, which was poured in a Petri dish (8cm). Wells (Ø: 2mm) were made by puncturing the agar with a plastic pipette. For the lytic assay, the samples were thawed on ice, centrifuged for 10min at 4°C at 13,000g, and 4µl of the supernatant was pipetted directly into the wells. Subsequently, the plates were incubated for 24h at 37°C. Total antibacterial activity was recorded as the degraded area around each sample-well, standardized by a serial dilution of chicken egg white lysozyme (Sigma) (2mg/ml, 1mg/ml, 0.750mg/ml, 0.500mg/ml, 0.250mg/ml, 0.125mg/ml, 0.062mg/ml, 0.031mg/ml).

Results and Discussion

Stress-resistance and immunity in workers

In Chapter one I report how worker ants respond to the joint influence of oral infection by bacteria and subsequent starvation. Although exposure to *S. marcescens* significantly increased mortality of the ants in both sets of the experiment, subsequent starvation had no influence on the rate at which the ants died from the infection, compared to uninfected ants (Figure 1, Table 1). In contrast, neither *P. entomophila* nor *E. coli* induced a significant decrease in survival when fed *ad libitum* (Figure 1, Table 1). This may indicate that these bacteria are not naturally pathogenic to *F. exsecta*, as was expected for *E. coli*. However, in the second set the ants showed prolonged survival during starvation, when they previously had been exposed to *E. coli* or *P. entomophila* (Figure 1, Table 1). This suggests a synergistic, beneficial effect of exposure to these bacteria on the starvation resistance of the ants. This improved starvation resistance after exposure to bacteria may have been induced by facultative anorexia (Adamo 2005; Adamo et al. 2007; Ayres & Schneider 2009). If the ants facultatively reduced food uptake upon infection, this may have increased their starvation resistance, which in turn may have improved the survival under starved conditions. This may also explain the observation that exposure to the pathogenic *S. marcescens* did not increase the mortality under starvation, as the general beneficial effect of exposure to bacteria may have canceled the detrimental effect of the dual stress. Alternatively, social immune defenses may have resulted in a beneficial effect on stress resistance. Ants often increase trophallaxis upon both starvation (Rueppell & Kirkman 2005) and infection (Hamilton et al. 2011). If this behavior benefits both starvation resistance and immune defenses, throphallaxis may result in a synergistic effect, and thus, reduce the joint effect of both stresses.

Table 1: *The influence of each factor (and interactions) on the survival of ants (Cox PH model), shown for both sets of the experiment separately. The hazard rate coefficient (β) indicates the change in the probability to die from the treatment/factor compared to the corresponding control treatment/factor. Two-way interactions indicate the change in the effect of the first factor on β due to the second factor. Starvation indicates the comparison of starved to ad libitum food conditions, and the bacteria names correspond to the effect of supplementing the food with S. marcescens, P. entomophila or E. coli, as compared to the control conditions. The first set was collected and performed in early July 2013 and the second set in late August 2013. Significant results are highlighted in bold.*

Treatment / Exposure	**Set**	**β ± se**	**χ^2**	**df**	**p-value**
Starvation	I	3.55±0.21	280.30	1.00	<0.0001
	II	2.46±0.2	150.14	1.00	<0.0001
S. marcescens	I	0.68±0.16	18.83	1.00	<0.0001
	II	0.73±0.17	18.63	1.00	<0.0001
E. coli	I	0.02±0.18	0.02	1.00	0.89
	II	0.11±0.19	0.33	1.00	0.56
P. entomophila	I	0.13±0.18	0.59	1.00	0.44
	II	0.21±0.18	1.35	1.00	0.25
Nest	I	N/A	148.81	9.79	<0.0001
	II	N/A	46.59	8.18	<0.0001
Starvation * *S. marcescens*	I	0.29±0.21	1.91	1.00	0.17
	II	-0.35±0.23	2.46	1.00	0.12
Starvation * *E. coli*	I	0.26±0.22	1.40	1.00	0.24
	II	-0.51±0.24	4.38	1.00	0.036
Starvation * *P. entomophila*	I	-0.14±0.22	0.38	1.00	0.54
	II	-0.6±0.24	6.20	1.00	0.013

Nevertheless, the beneficial effect of exposure to *E. coli* and *P. entomophila* was only observable in the second set of the experiment. Furthermore, the response of the ants in terms of gene expression differed between the two sets (Table 2). In the first set, the ants did not respond to any of the exposures when fed *ad libitum*, and down-regulated gene expression upon starvation, unless they had been exposed to *S. marcescens*. In the second set, the ants down-regulated gene expression when they were *ad libitum* exposed to *S. marcescens*, but did not respond to starvation after any of the exposures. This may indicate temporal variation in the physiological response to the stresses. The origin of this variation may be seasonal differences in the environmental conditions. However, it is more likely that demographic changes within the colony resulted in differences between the two sets. In *F. exsecta*, a first

Table 2: *Results from linear mixed effects models on the effect of Exposure and Starvation on gene expression.*

Treatment / Exposure	**β ± se**	**t**	**adj. p**	**β ± se**	**t**	**adj. p**	**β ± se**	**t**	**adj. p**	**β ± se**	**t**	**adj. p**
	Aryl			**Vg1**			**IR1**			**IR3**		
E. coli	-0.81±0.90	-0.901	0.99	-0.23±1.06	-0.217	0.99	0.50±0.79	0.628	0.99	-0.01±0.73	-0.011	0.99
Serratia	-0.24±0.90	-0.266	0.9	1.96±1.06	1.846	0.35	0.06±0.79	0.074	0.94	-0.84±0.73	-1.145	0.51
Pseudomonas	-2.42±0.93	-2.618	0.11	-1.67±1.09	-1.534	0.29	0.02±0.82	0.03	0.98	-0.76±0.75	-1.006	0.53
Starvation	4.27±0.90	4.745	<0.0001	5.44±1.06	5.13	<0.0001	4.80±0.79	6.052	<0.0001	5.11±0.73	6.997	<0.0001
E. coli * Starvation	0.59±1.24	0.48	0.7	-1.17±1.46	-0.804	0.61	-1.56±1.09	-1.427	0.6	-1.26±1.00	-1.251	0.6
Serratia * Starvation	-5.43±1.27	-4.274	0.0001	-8.96±1.50	-5.979	<0.0001	-6.62±1.12	-5.902	<0.0001	-7.11±1.03	-6.894	<0.0001
Pseudomonas * Starvation	0.44±1.29	0.343	0.85	0.47±1.52	0.307	0.85	-1.38±1.14	-1.214	0.46	-1.55±1.05	-1.484	0.43
	PPO			**Hyme**			**LPS-bp**			**LysC**		
E. coli	-0.04±0.94	-0.045	0.99	0.79±1.36	0.578	0.99	-1.08±1.10	-0.988	0.99	-0.33±0.64	-0.524	0.99
Serratia	-1.40±0.94	-1.49	0.41	1.92±1.36	1.412	0.41	-2.15±1.10	-1.96	0.35	0.18±0.64	0.279	0.9
Pseudomonas	-1.42±0.97	-1.473	0.29	2.49±1.40	1.775	0.27	-2.50±1.13	-2.212	0.15	-0.57±0.66	-0.873	0.55
Starvation	5.95±0.94	6.338	<0.0001	7.56±1.36	5.551	<0.0001	4.35±1.10	3.961	0.0002	5.11±0.64	8.001	<0.0001
E. coli * Starvation	-2.00±1.29	-1.55	0.6	-1.02±1.87	-0.546	0.7	0.35±1.51	0.232	0.82	-0.89±0.88	-1.011	0.6
Serratia * Starvation	-6.45±1.33	-4.854	<0.0001	-5.01±1.92	-2.602	0.0116	-4.29±1.55	-2.764	0.0084	-7.61±0.90	-8.43	<0.0001
Pseudomonas * Starvation	-1.87±1.35	-1.385	0.43	-3.45±1.95	-1.768	0.43	-0.15±1.58	-0.095	0.93	-1.52±0.92	-1.659	0.43
	β1,3g			**Toll**								
E. coli	-0.71±0.92	-0.766	0.99	-0.14±0.77	-0.188	0.99						
Serratia	-0.23±0.92	-0.245	0.9	-0.48±0.77	-0.626	0.89						
Pseudomonas	-0.65±0.95	-0.682	0.6	-0.49±0.79	-0.623	0.6						
Starvation	6.54±0.92	7.072	<0.0001	4.83±0.77	6.298	<0.0001						
E. coli * Starvation	-1.18±1.27	-0.925	0.6	-1.19±1.06	-1.128	0.6						
Serratia * Starvation	-4.99±1.31	-3.816	0.0004	-4.37±1.08	-4.03	0.0002						
Pseudomonas * Starvation	-0.87±1.33	-0.655	0.8	-0.64±1.10	-0.583	0.8						

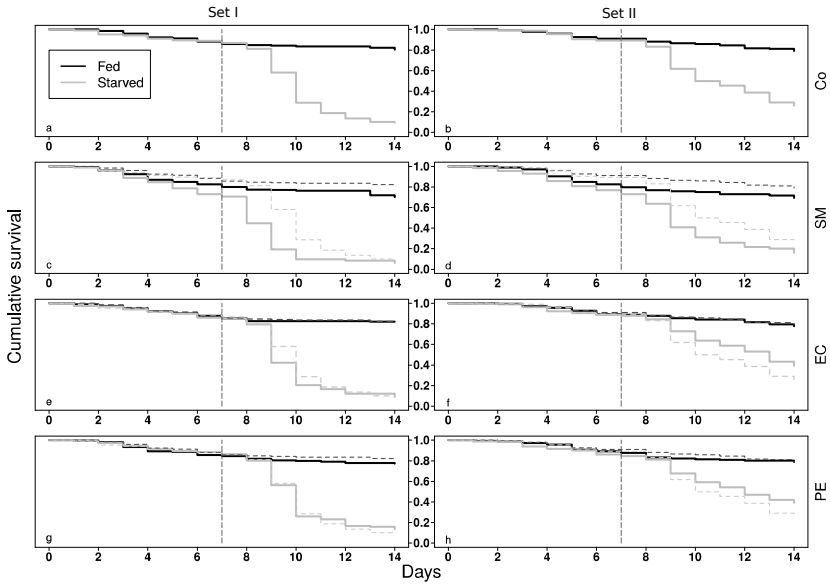

Figure 1: *Cumulative survival after exposure to food supplemented with LB medium (Co), S. marcescens (SM), E. coli (EC), or P. entomophila (PE). The ants were either fed daily ad libitum throughout the experiment (black lines), or for a limited time followed by starvation (gray lines). The vertical dashed line indicates the onset of starvation for the starvation treatment. For each bacteria-supplemented diet, the survival of the control diet is indicated with fine dashed lines.*

worker cohort, which ecloses in mid-July, is raised by overwintered workers from the previous year. A second worker cohort, to eclose in late August, is then raised by workers from the first cohort. As the first set was collected early July, this set encompassed overwintered workers. The second set, collected in late August, encompassed a mixture of workers from the first and second worker cohort of the season. Given that older workers of many eusocial insects show immune-senescence in the form of lowered immune responses (Doums et al. 2002; Laughton et al. 2011), differences in the average age of the ants may have resulted int the differential responses to *S. marcescens* between the two sets. Similarly, the younger individuals also may have been more resilient to starvation, which may explain the absent response to starvation in the second set in terms of gene expression.

Inbreeding and stress-resistance in workers

In Chapter two I report how inbreeding can influence the mechanisms of stress-resistance and immune defenses. Exposure to reactive oxygen species (ROS) significantly reduced lytic activity of the hemolymph (GLMM

[Diet]: z = 23.12, p = 0.002). Furthermore, workers from inbred colonies showed a significantly stronger antibacterial activity compared to outbred colonies, however, only when the ants received a diet supplemented with ROS (GLMM [HL]: z = 22.786, p = 0.005). The ROS-rich diet had a general negative effect on the ants' survival (Survreg [Diet]: z = 23.921, p < 0.001). This indicates that inbred individuals compensate for the increased oxidative stress by up-regulating immune defenses. Thus, the resistance to oxidative stress and immune defenses may be subject to a trade-off only in inbred individuals. The survival did not differ between inbred and outbred ants, neither under control conditions, nor under nutritional stress (Survreg [Diet]: z = 21.169, p = 0.24; Survreg [Diet*HL]: z = 22.786, p = 0.005). Thus, in terms of survival, such a trade-off may only become evident when several stresses are present. This suggests that inbreeding can create hidden variation in stress-resistance across colonies, which could only manifest negatively when several stresses are present. Nevertheless, the results in Chapter one indicated a remarkably strong resistance of *F. exsecta* workers against multiple stresses. This may be related to the polygynous structure of the Prästkulla colonies investigated in Chapter one. As polygyny increases the genetic variation (Dekoninck et al. 2014), these colonies likely resembled outbred colonies. Therefore, these results indicate that genetic variation may be an important factor mediating trade-offs in stress-resistance.

Variation in immune defenses across castes

In Chapter three I investigated how young queens, young males, indoor workers (nurses) and outdoor workers (foragers) differ in their constitutive and induced immune defenses upon infection by the fungus *B. bassiana*. Queens and males clearly differed in their constitutive expression of immune genes, as well as in their response in gene expression to the infection (Figure 2, Tables 3 & 4). Due to their short lifespan, males are expected to invest less into immune defenses than queens. Yet, in general, males did not show lower constitutive expression of all immune genes than queens. In fact, an antifungal recognition protein (*β1,3g*) and an antimicrobial peptide (*Hyme*) were more expressed in males than queens

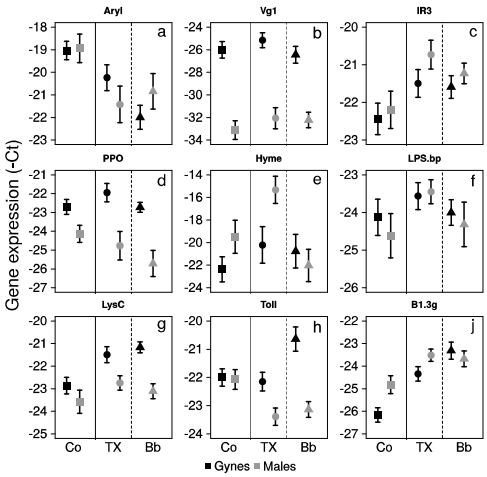

Figure 2: *Gene expression levels for queens (black) and males (gray), shown as inverted Ct values (higher values indicate higher expression levels). Gene expression was measured for the constitutive state (Co - squares), four days after treatment with Triton-X (TX - circles), and four days after exposure to B. bassiana (Bb - triangles). The top three genes (panels a-c) were classified as pleiotropic genes, and the lower six genes (panels d-j) were classified as immune genes. Error bars show the 95% confidence intervals across nests.*

(Figure 2, Table 3). In particular the gene Hymenoptaecin appears to be frequently male biased (Colgan et al. 2011; Koch et al. 2013), possibly because Imd-pathway-induced genes are in general often biased towards males (Schmid-Hempel 2005). A similar pattern emerged in the constitutive gene expression of the workers. The nurses showed a generally higher constitutive gene expression than the foragers, except for the genes *β1,3g* and *Hyme*, of which *Hyme* was constitutively more expressed in foragers (Figure 3, Table 3). Given that foragers are older than nurses, the bias of *Hyme* towards short lived males and old foragers may reflect

Table 3: *Results from gene-by-gene linear mixed effects models on the difference in constitutive gene expression between the reproductive castes, or the worker-classes. The average difference between reproductives (Males vs Queens) and workers (Foragers vs Nurses) in constitutive gene expression (inverse Ct values) is given as the parameter estimates plus/minus standard error (β±SE). All p-values within each caste-group were FDR corrected.*

	Reproductives, 2013			**Workers, 2015**		
	β±SE	**t-value**	**adj. p-value**	**β±SE**	**t-value**	**adj. p-value**
Aryl	0.09±0.36	0.25	0.8	-3.87±0.35	-10.99	<0.0001
Vg1	-7.06±0.40	-17.47	<0.0001	-4.49±0.71	-6.35	<0.0001
IR3	0.24±0.29	0.84	0.53	-0.86±0.33	-2.61	0.0173
PPO	-1.43±0.29	-4.95	0.0001	-1.68±0.34	-4.93	0.0005
Hyme	2.89±0.80	3.6	0.0038	3.20±0.81	3.94	0.0009
LPS-bp	-0.51±0.34	-1.52	0.22	-1.65±0.43	-3.89	0.0021
LysC	-0.71±0.29	-2.44	0.0427	-0.72±0.30	-2.43	0.0306
Toll	-0.07±0.22	-0.32	0.8	-1.12±0.39	-2.84	0.0112
β1,3g	1.37±0.22	6.16	<0.0001	-0.31±0.21	-1.44	0.17

increased investment into the Imd pathway in individuals with low residual life expectancy.

In terms of survival, queens and males did not show a significant difference in their susceptibility to the infection (Survreg [$Caste_{Infected}$]: β±SE = -0.08±0.05, z = 1.73, p = 0.08). Although the susceptibility to *B. bassiana* was also found not to be different between queens and males in other ant species (Ho & Frederickson 2014), it is possible that this result originated from a lack of resolution in the survival analysis. Similarly, nurses and foragers did not show significant differences in the survival upon infection (Survreg [$Class_{Infected}$]: β±SE = -0.03±0.03, z = 1.11, p=0.27), which, however, may again relate to the rapid decrease in survival after infection due to the high dose of infection.

Upon infection, the queens and nurses responded to the infection by up-regulating the two antifungal defense genes *β1,3g* and *Toll*, whereas males and foragers did not show such a response (Figure 2, Table 4). This suggests that queens and nurses exhibit stronger induced immune defenses towards fungal infections than males or foragers, respectively. In the queens this induced immune defense came along with decreased expression of the two storage proteins *Aryl* and *Vg1*, as well as the general immune gene *PPO* (Figure 2, Table 4). This suggests a cost to the antifungal immune defense, and reflects the expectation that queens

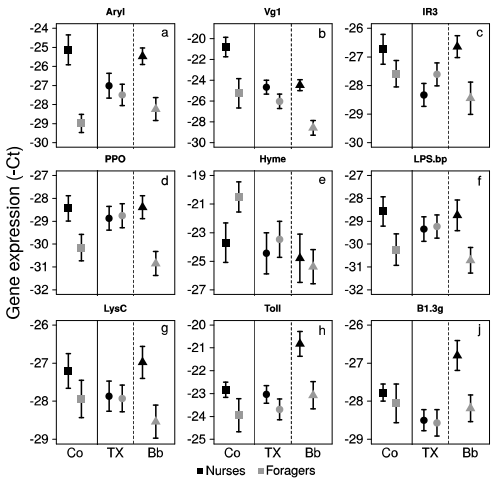

Figure 3: *Gene expression levels for nurses (black) and foragers (gray), shown as inverted Ct values (higher values indicate higher expression levels). Gene expression was measured for the constitutive state (Co - squares), four days after treatment with Triton-X (TX - circles), and four days after exposure to B. bassiana (Bb - triangles). The top three genes (panels a-c) were classified as pleiotropic genes, and the lower six genes (panels d-j) were classified as immune genes. Error bars show the 95% confidence intervals across nests.*

face trade-offs between long term interests, such as colony-foundation and longevity, or short-term survival. In the males, the infection resulted in a down-regulation of the antibacterial immune genes *Hyme* and *LPS-bp*, and – like in the queens – the general immune gene *PPO* (Figure 3, Table 4). The foragers showed a similar response, but down-regulated all genes except *β1,3g* and *Toll* (Figure 4, Table 4). This suggests that males and foragers down-regulate genes that are not directly involved in the antifungal immune response.

In combination, these results support the expectation that males and foragers invest less into immune defenses than queens or nurses, respectively. This may originate from a general mediating effect of age related life histories on immune defenses. As males have only a short life

Table 4: *Results from gene-by-gene linear mixed effects models on the effect of Caste/Class, Treatment (Beauveria vs TritonX) and the Caste/Class by Treatment interaction on induced gene expression. Interaction indicates the Caste by Treatment interaction. Parameter estimates are given as β plus/minus standard error (SE). All p-values within each caste-group were FDR corrected.*

	Caste/Class			**Treatment**			**Interaction**		
	β±SE	**t**	**adj. p**	**β±SE**	**t**	**adj. p**	**β±SE**	**t**	**adj. p**
Reproductives, 2013									
Aryl	-1.15±0.39	-2.96	0.0054	-1.76±0.37	-4.76	0.0001	2.29±0.53	4.31	0.0005
Vg1	-6.77±0.45	-15.12	<0.0001	-1.28±0.43	-2.99	0.0108	1.11±0.62	1.79	0.12
IR3	0.77±0.22	3.48	0.0012	-0.09±0.22	-0.42	0.68	-0.41±0.32	-1.28	0.24
PPO	-2.86±0.32	-9.04	<0.0001	-0.78±0.30	-2.61	0.0231	-0.17±0.43	-0.39	0.7
Hyme	4.87±0.94	5.18	<0.0001	-0.56±0.94	-0.59	0.63	-6.13±1.35	-4.53	0.0003
LPS-bp	0.12±0.24	0.51	0.61	-0.44±0.23	-1.91	0.0939	-0.42±0.33	-1.27	0.24
LysC	-1.26±0.19	-6.49	<0.0001	0.32±0.19	1.7	0.13	-0.68±0.27	-2.49	0.0317
Toll	-1.24±0.23	-5.52	<0.0001	1.51±0.23	6.69	<0.0001	-1.25±0.32	-3.87	0.0006
β1,3g	0.83±0.21	3.87	0.0004	1.03±0.21	4.81	0.0001	-1.19±0.31	-3.87	0.0006
Workers, 2015									
Aryl	-0.48±0.27	-1.76	0.19	1.55±0.27	5.68	<0.0001	-2.30±0.39	-5.91	<0.0001
Vg1	-1.36±0.40	-3.41	0.0108	0.20±0.40	0.49	0.63	-2.74±0.57	-4.81	<0.0001
IR3	0.72±0.25	2.92	0.0225	1.69±0.25	6.83	<0.0001	-2.55±0.35	-7.22	<0.0001
PPO	0.11±0.29	0.39	0.77	0.48±0.29	1.68	0.13	-2.58±0.41	-6.26	<0.0001
Hyme	0.97±0.68	1.42	0.29	-0.36±0.68	-0.52	0.63	-1.57±0.97	-1.61	0.11
LPS-bp	0.11±0.30	0.37	0.77	0.60±0.30	1.98	0.0791	-2.10±0.43	-4.89	<0.0001
LysC	-0.06±0.21	-0.3	0.77	0.89±0.21	4.3	0.0001	-1.49±0.29	-5.07	<0.0001
Toll	-0.66±0.32	-2.05	0.14	2.21±0.32	6.82	<0.0001	-1.57±0.46	-3.41	0.0014
β1,3g	-0.07±0.21	-0.33	0.77	1.70±0.21	8.24	<0.0001	-1.31±0.29	-4.45	0.0001

expectancy, and foragers also usually have only a short residual life expectancy, it is possible that residual life expectancy plays a major, general role in the mediation of immune defenses among the castes in social insect societies. This is also supported by the observation the the Imd pathway related gene *Hyme* was biased towards males and foragers, as the Imd pathway has been shown to be connected to age related life histories (Myllymäki et al. 2014).

Variation in reaction norms to temperature- and humidity-stress

Chapter four reports how workers from two populations, Furuskär and Prästkulla, differ in their response to different temperature and humidity regimes. After ten days on the experimental conditions, gene expression was overall higher at 20°C than 25°C, except at 75% humidity in the Furuskär population (Figure 4, Tables 5 & 6). This is consistent with the frequently observed increase of gene expression in colder environments in ectotherms (Ju et al. 2002; McClelland et al. 2006), and likely reflects compensation in gene expression due to reduced metabolic activity (Clarke & Fraser 2004; Vogt & Appel 1999). Furthermore, this result may reflect resistance to desiccation by reduced gene expression, and thus, reduced water consumption in protein synthesis, in the warmer environment (Teets et al. 2012).

Table 5: *Results from linear mixed effects models on the selected principal components capturing gene expression after ten days at the experimental conditions. Temp indicates the effect of temperature (cold vs hot), Hum the effect of humidity (dry vs moist) and Pop the populations (Island vs Mainland). All p-values were adjusted for false discovery rates.*

	PC1			PC2		
	SS	**F-value**	**adj. p**	**SS**	**F-value**	**adj. p**
Temp	14.97	$F_{1,220.66}$ =57.91	<0.0001	0.09	$F_{1,220.72}$= 0.26	0.61
Hum	0.54	$F_{1,220.51}$= 2.10	0.15	4.14	$F_{1,220.59}$ = 11.77	0.0014
Pop	65.47	$F_{1,19.04}$= 253.30	<0.0001	45.81	$F_{1,19.21}$= 130.10	<0.000
Temp:Hum	0.00	$F_{1,220.66}$= 0.00	0.97	1.10	$F_{1,220.72}$= 3.11	0.16
Pop:Temp	3.71	$F_{1,220.66}$ = 14.36	0.0004	1.50	$F_{1,220.72}$= 4.27	0.0400
Pop:Hum	0.46	$F_{1,220.51}$= 1.80	0.18	1.88	$F_{1,220.59}$= 5.35	0.0433
Pop:Temp:Hum	5.47	$F_{1,220.66}$ = 21.15	<0.0001	6.49	$F_{1,220.72}$ = 18.44	<0.0001

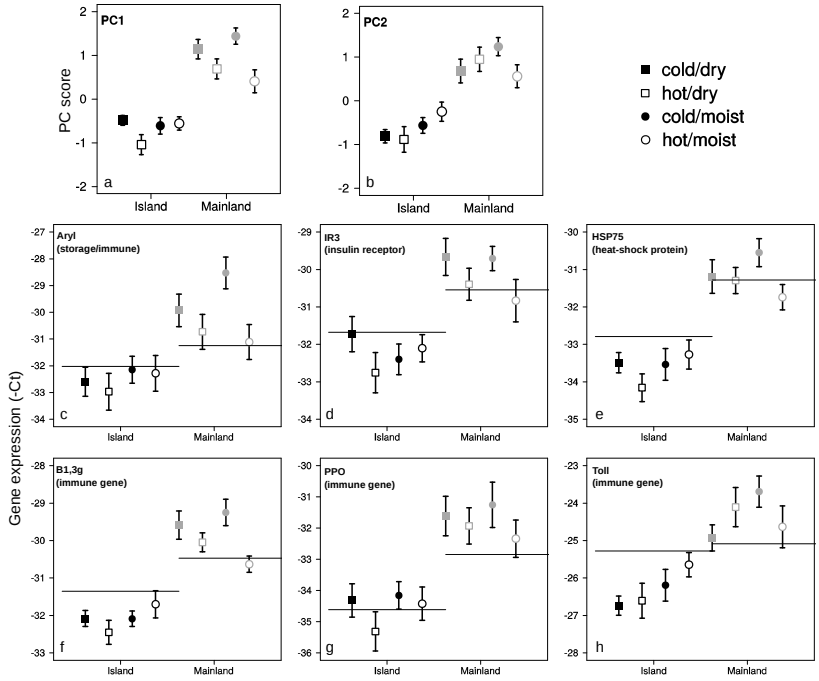

Figure 4: *PC scores (panels a & b) representing gene expression of six genes (panels c-h) after ten days acclimatization to one of four laboratory conditions. Squares indicate gene expression at dry conditions, and circles indicate the gene expression at humid conditions. Filled symbols represent gene expression at cold temperatures, and open symbols represent gene expression at hot temperatures. Error bars indicate 95% confidence intervals.*

A role of gene expression in desiccation resistance was also reflected in the response to the two humidities. Ants from both populations showed higher levels of gene expression at 75% than 50% humidity, but for Furuskär this effect occurred only at 20°C, and for Prästkulla only at 25°C (Figure 4, Tables 5 & 6). This may again reflect increased desiccation resistance in the dryer environment due to reduced gene expression (Teets et al. 2012). In addition, at 25°C the Prästkulla population showed the opposite pattern, with an overall lower level of gene expression at 75% humidity (Figure 4, Tables 5 & 6). This indicates variation in how worker ants from the two populations respond to environmental conditions.

Such variation between the populations was also visible in survival, which was significantly shorter at 25°C than 20°C at 50% humidity, but only for ants from Furuskär (CoxPH [Temp]: $X^2 = 89.6$, $p<0.0001$; CoxPH [Temp*Pop]: $X^2 = 11.6$, $p<0.0001$). As the high temperature may lead to faster desiccation at the dry conditions, this likely reflects desiccation-

stress, but only in ants from Furuskär. This is also reflected in the gene expression response to the experimental conditions in comparison with the pre-experiment level of gene expression. After ten days at 25°C and 50% humidity, ants from Furuskär showed reduced expression of all genes than before the experiment (Figure 4, Table 6). In contrast, ants from Prästkulla only up-regulated one gene (Toll) under these conditions, but instead up-regulated all genes at 20°C and 75% humidity (Figure 4, Table 6). Thus, the ants from the two populations not only responded to different conditions, but also in opposite directions. As the Prästkulla population already showed higher gene expression levels before the experiment, this increased the differences between the populations even further. This suggests that the level of gene expression at a particular

Table 6: *Results from linear mixed effects models on the expression of each gene after ten days at the experimental conditions. Temp indicates the effect of temperature (cold vs hot), Hum the effect of humidity (dry vs moist) and Pop the populations (Island vs Mainland). All p-values were adjusted for false discovery rates.*

	SS	F-value	adj. p	SS	F-value	adj. p	SS	F-value	adj. p
	Aryl (storage/immune gene)			**IR3 (insulin receptor)**			**HSP75 (heat-shock protein)**		
Pop	83.35	$F_{1,19.02}$= 36.65	<0.0001	148.56	$F_{1,19.17}$= 101.24	<0.0001	148	$F_{1,19.25}$= 155.65	<0.0001
Temp	58.62	$F_{1,220.38}$= 25.77	<0.0001	25.5	$F_{1,220.95}$= 17.38	0.0001	10.79	$F_{1,220.85}$= 11.35	0.0012
Hum	17.14	$F_{1,220.29}$= 7.54	0.0197	0.94	$F_{1,220.76}$= 0.64	0.43	4.16	$F_{1,220.7}$= 4.37	0.0753
Pop:Temp	33.51	$F_{1,220.38}$= 14.73	0.0005	4.76	$F_{1,220.95}$= 3.24	0.12	2.89	$F_{1,220.85}$= 3.04	0.12
Pop:Hum	0.12	$F_{1,220.29}$= 0.05	0.82	0.73	$F_{1,220.76}$= 0.5	0.58	1.41	$F_{1,220.7}$= 1.49	0.336
Temp:Hum	9.8	$F_{1,220.38}$= 4.31	0.12	3.07	$F_{1,220.95}$= 2.09	0.3	0.08	$F_{1,220.85}$= 0.09	0.92
Pop:Temp:Hum	17.13	$F_{1,220.38}$= 7.53	0.0079	11.18	$F_{1,220.95}$= 7.62	0.0079	15.04	$F_{1,220.85}$= 15.82	0.0003
	B1,3g (immune gene)			**PPO (immune gene)**			**Toll (immune gene)**		
Pop	100.36	$F_{1,19.24}$= 177.96	<0.0001	326.73	$F_{1,19.21}$= 134.73	<0.0001	126.78	$F_{1,19.36}$= 105.71	<0.0001
Temp	12.3	$F_{1,220.74}$= 21.81	<0.0001	26.92	$F_{1,221.17}$= 11.1	0.0012	1.24	$F_{1,221.12}$= 1.04	0.31
Hum	0.88	$F_{1,220.61}$= 1.57	0.26	3.7	$F_{1,220.94}$= 1.53	0.26	18.37	$F_{1,220.93}$= 15.32	0.0007
Pop:Temp	12.81	$F_{1,220.74}$= 22.71	<0.0001	0.1	$F_{1,221.17}$= 0.04	0.84	2.3	$F_{1,221.12}$= 1.92	0.2
Pop:Hum	3.69	$F_{1,220.61}$= 6.55	0.0669	4.57	$F_{1,220.94}$= 1.88	0.34	2.41	$F_{1,220.93}$= 2.01	0.34
Temp:Hum	0.09	$F_{1,220.74}$= 0.17	0.92	0.01	$F_{1,221.17}$= 0.00	0.96	6.64	$F_{1,221.12}$= 5.54	0.12
Pop:Temp:Hum	10.33	$F_{1,220.74}$= 18.32	0.0002	8.67	$F_{1,221.17}$= 3.58	0.0599	17.55	$F_{1,221.12}$= 14.63	0.0003

environmental condition is not only determined by the condition itself. Instead, different reaction norms of the populations may have resulted in the differential responses, despite similar environmental conditions.

In conclusion, these results show variation in how individuals from different populations respond to environmental conditions. This variation likely reflects different reaction norms, due to the different habitat of the populations. The Furuskär populations are mostly located in a predominantly warm and dry environment, whereas Prästkulla constitutes a shady, moist forest. Given that the differentially regulated genes in this study also included immune genes, this suggests that reaction norms can also affect traits not directly involved in the response to the specific conditions. As a consequence, changes in environmental conditions may not only affect the resistance to environmental stresses, but may also generate variation in other traits, such as immune defenses.

Conclusions and Perspectives

In this thesis I investigated the stress responses of the ant *F. exsecta* in the light of host-parasite interactions. The results of the separate thesis chapters clearly indicate that ecological conditions and stresses have an impact on the immune defenses of the ants, and thus, may influence the outcome of host-parasite interactions. The findings presented in chapters three and four show vast variation in stress and immune responses between populations and even within colonies as a result of ecological factors. Such variation due to environmental conditions or the adopted task of an individual within a colony may strongly influence the evolutionary trajectory of the species. For example, the environmental conditions encountered in the Prästkulla populations have changed over the last decades, as this former logging site was allowed to grow over by the forest. This may have resulted in a purge of the less resilient, monogyne colonies, and thus led to the predominance of polygyne colonies at this site. This may also result in reduced levels of inbreeding within the colonies, and

thus, alter the susceptibility to the joint effects of oxidative stress and immune defenses presented in chapter two. However, this effect still has to be investigated for this population. Similarly, the effect of caste on immune defenses was only investigated for monogyne colonies, yet, given the consistent patterns, it is likely that the results reported in chapter three also apply to polygyne colonies. In conclusion, this thesis provides insight into the impact of ecological factors on the resistance and responses to stress in a social insect. Yet, as host-parasite interactions always involve two parties, - the parasite and the host – the results in this thesis present only one side of the coin. An important next step would be to investigate if, and how, the parasites respond to stressed hosts. If the parasites are indeed able to detect and adapt to different stresses the host experiences, this could directly influence and/or initiate the co-evolution of *F. exsecta* and its parasites.

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