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# Social isolation causes downregulation of immune and stress response genes and behavioural changes in a social insect

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## Abstract

Humans and other social mammals experience isolation from their group as stressful, triggering behavioural and physiological anomalies that reduce fitness. While social isolation has been intensely studied in social mammals, it is less clear how social insects, which evolved sociality independently, respond to isolation. Here we examined whether the typical mammalian responses to social isolation, e.g., an impaired ability to interact socially and immune suppression are also found in social insects. We studied the consequences of social isolation on behaviour and brain gene expression in the ant *Temnothorax nylanderi*. Following isolation, workers interacted moderately less with adult nestmates, increased the duration of brood contact, and reduced the time spent self-grooming, an important sanitary behaviour. Our brain transcriptome analysis revealed that only a few behaviour-related genes had altered their expression with isolation time. Rather, many genes linked to immune system functioning and stress response had been downregulated. This probably sensitizes isolated individuals to various stressors, in particular because isolated workers exhibit reduced sanitary behaviour. We provide evidence of the diverse consequences of social isolation in social insects, some of which resemble those found in social mammals, suggesting a general link between social well-being, stress tolerance, and immune competence in social animals.

## KEYWORDS

immune suppression, social insects, stress, *Temnothorax*, transcriptomics

## 1 | INTRODUCTION

Social isolation is a topic of increasing relevance for public health, particularly regarding an aging human population, as the negative effects of isolation are more prevalent later in life (Courtin & Knapp, 2017; Nicholson, 2012). Moreover, the effects of social isolation have gained increasing importance recently due to the quarantine measures being taken to avoid the spread of novel infectious diseases. A strong reduction in social contacts presents a mortality risk, due to its positive association with high blood pressure, high cholesterol

levels, smoking, self-neglect, and cognitive decline (Brennan et al., 2019; Cacioppo et al., 2011; Pantel et al., 2013). Social isolation has been defined in many ways, with the most general one relating to suffering from reduced contact with group members compared to the desired level (Hawkey & Capitanio, 2015). Not only humans experience the negative consequences of social isolation, but so do other animals with a social lifestyle. It has been proposed that the strength of response to social isolation may even assist to characterize a species' level of sociality or interdependence of members of a social group (Lihoreau et al., 2009; Wang et al., 2016).

Owing to its relevance for humans, most research on social isolation has been conducted on small mammals, like rats and mice, as well as on mammals resembling humans in their social organization, such as prairie voles. With isolation, social mammals become more aggressive towards unfamiliar conspecifics, demonstrate less interest in social interactions, and may fail to recognize their own group members (Lukkes et al., 2009; Wise, 1974; Zhao et al., 2009). The absence of social interactions in social mammals can also reduce boldness and risk-taking, increase neophobia, and impede learning processes (Fone & Porkess, 2008; Grippo et al., 2008; Pan et al., 2009). While some experiments have demonstrated an increase in physical activity with isolation, others have reported no change or even a decrease (Grippo et al., 2008; Silva-Gomez et al., 2003; Weiss et al., 2004). The behavioural responses following isolation are correlated with shifts in physiology and brain gene expression. The levels of some stress-related hormones, such as cortisol, increase with isolation in mammals; while others, such as corticosterone, exhibit no clear pattern (Hawkey et al., 2012; Kanitz et al., 2004; Lukkes et al., 2009). Social isolation is associated with the activity of the hypothalamic-pituitary-adrenocortical axis, which regulates the mammalian response to stress (Cacioppo et al., 2015). The absence of social contacts also causes suppression of the immune system, which in turn can lead to a higher risk of cancer and chronic inflammation (Cruces et al., 2014; Hawkey et al., 2012; Wu et al., 2000).

Changes in the expression of genes related to stress response and anxiety behaviours have also been linked to social isolation. For example, in prairie voles, isolation leads to the overexpression of corticotrophin-releasing factor, vasopressin, and oxytocin in the hypothalamus. The latter two are sociality-related genes that are also linked to anxiety-related behaviours (Pan et al., 2009). In male mice, early social isolation leads to motor hyperactivity and behavioural disinhibition, caused by a robust downregulation of serotonin receptors in the brain (Bibancos et al., 2007). Female mice with a history of social isolation, in contrast, exhibit higher levels of anxiety and upregulation of brain-derived neurotrophic factors in the cerebral cortex (Kumari et al., 2016).

Sociality has repeatedly evolved not only in mammals but also in insects, with eusocial insects exhibiting the highest level of social organization. Social insects demonstrate a division of labour between queens and workers, and often also between workers of different castes, such as nurses and foragers. Social isolation has severe consequences for social insects. Isolated social insect workers die faster than grouped ones, due to lacking those resources regularly provided by nestmates and to the general stress induced by isolation, resulting in faster respiration, faster depletion of body reserves, and/or inefficient digestion (Boulay et al., 1999; Koto et al., 2015; Modlmeier et al., 2013; Szczuka et al., 2011). Social isolation also modifies behaviour. Aggression may either increase or decrease with isolation, probably due to changes in the cuticular hydrocarbon profile that is important for nestmate recognition, or in the brain structure (corpora allata size) of isolated workers (Breed, 1983). For example, while aggression towards (former) nestmates may increase

with the duration of isolation, the aggression against non-nestmates decreases (Boulay & Lenoir, 2001; Lenoir et al., 2001). Antennation between reunited workers following short isolation decreases, whereas trophallaxis (social food exchange) increases (Boulay et al., 1999; Cybulska et al., 2000; Korczyńska et al., 2005; Wagner-Ziemka et al., 2008). When social insects are isolated during development, their developmental and/or behavioural maturation is impaired, leading to slower growth of the mushroom body and/or a reduction in brood-care activity (Champalbert & Lachaud, 1990; Seid & Junge, 2016). Finally, the presence of conspecifics can moderate or buffer the stress induced by competitors or predators (Bowen et al., 2013; Tian et al., 2017). The mechanisms of molecular response to social isolation have rarely been studied in insects, compelling us to determine whether the phenotypic responses to isolation are regulated via similar molecular pathways in insects and mammals. Certain behaviours, such as novelty-seeking or responses to social stimuli, are controlled via similar molecular pathways in honeybees and humans, suggesting deep evolutionary conservation of some genes (Liang et al., 2012; Shpigler et al., 2017).

We focused on the ant *Temnothorax nylanderi*, which dwells in colonies of a few dozen workers on average, and sought to examine changes in worker phenotypes triggered by different periods of isolation. Isolated workers of *T. nylanderi* demonstrated increased mortality and lower body reserves at death compared to grouped workers (Modlmeier et al., 2013). We hypothesized that social isolation would affect behaviour and gene expression and that these effects would resemble and be mechanistically similar to stress-induced responses. We predicted worker activity and their tendency to move along the arena's edges to first increase and then decrease with isolation, as workers initially search for their lost colony, but later reduce activity when failing to find it or to escape from the arena. Because social stress changes self-grooming patterns (Denmark et al., 2010), and self-grooming is an important aspect of social immunity in social insects (Theis et al., 2015), we predicted that this hygienic behaviour would decline with isolation. Isolated social mammals demonstrate less interest in social interactions following their return to the group (Lukkes et al., 2009; Wise, 1974; Zhao et al., 2009). We anticipated reduced contacts with nestmates following a lengthy period of isolation, but more frequent contacts with nestmates following a shorter period of isolation (a hump-shaped pattern). We predicted that the time allocated to tending the nestmate brood would decline, together with a general decline in social behaviours with isolation, but we did not base our expectation on concrete evidence from prior studies. In respect to brain gene expression, we predicted social isolation to modify the expression of genes controlling those behaviours that change with isolation, such as general care behaviours (towards the brood, queen, or nestmates), which are regulated via, e.g., *vitellogenin-like A* in *Temnothorax* ants (Kohlmeier et al., 2018). We also predicted that the regulation of immune genes would be altered, as immune suppression is a characteristic consequence of isolation in other social animals

(Hawkey et al., 2012). It is difficult to foresee the direction of change. While some genes involved in immune system functioning are downregulated with social isolation in humans, other genes are upregulated (Cole et al., 2007). Finally, we predicted that those genes related to improving tolerance to stressors, such as oxidative stress, would be downregulated, as isolation makes social animals more vulnerable to stress (Zlatković, Bernardi, et al., 2014; Zlatković, Todorović, et al., 2014).

## 2 | MATERIALS AND METHODS

### 2.1 | Ant collection and maintenance

*Temnothorax nylanderi* establishes colonies of a few dozen workers, which inhabit cavities in acorns and sticks on the floor of European deciduous forests. Colonies contain a single queen and may occupy more than one nest (they are monogynous and polydomous; Kramer et al., 2014). We collected 14 queenright colonies in December 2018 from Lennebergwald, near Mainz, Germany, and brought them to the laboratory ( $129 \pm 24$  workers per colony; mean  $\pm$  1 SD; at least 30 larvae in each colony). Each colony was kept in an artificial nest ( $50 \times 10 \times 3$  mm length  $\times$  width  $\times$  height), placed inside a box with a plaster floor, and divided into three chambers. Water, half a cricket, and a droplet of honey were provided once a week. The colonies were kept in a climate cabinet at 21°C and a photoperiod of 12:12 h light:dark cycle.

### 2.2 | Isolation experiment and behavioural measurements

The experiment took place in January–March 2019. Nurses, young workers (<1-year-old) tasked with brood care, were individually isolated from 14 focal colonies for increasing durations: no isolation or 0 h (control), 1 h, 24 h (1 day), 168 h (7 days), or 672 h (28 days). For each colony, the experiment started on a different day, between 21 and 58 days post collection (Appendix S1). Isolated workers were placed individually in Petri dishes (5 cm diameter), each containing two tiny plates ( $d = 0.5$  cm) with water and honey, which were replaced twice weekly. For each isolation duration, seven workers per colony were used, resulting in 35 workers per colony times 14 colonies, totalling 490 workers. We compared the body mass of workers isolated for different durations (one worker per colony and isolation duration, five workers in total per colony), but found no changes in mass with isolation duration ( $p > 0.20$ ). The following three behaviours were recorded from the same ant in the Petri dish of each focal worker: activity; tendency to move along the arena's edges (hereafter, edge preference); and time spent self-grooming. The activity was measured by video-recording the isolated worker for 10 min and summing the time it was active/moving. Edge preference was measured by taking 60 screenshots, one every 10 s, of the 10 min. video, measuring the distance of the worker from

the edge, and calculating the average distance in all screenshots. Self-grooming was measured by documenting the time (for 5 min) the worker was cleaning itself. Three additional behaviours were recorded from different ants following the introduction of either a nestmate larva, a dead nestmate worker, or a dead non-nestmate worker into the isolation arena (the Petri dish): the total time the worker spent in contact with larvae, contact duration with a nestmate and contact duration with a non-nestmate conspecific. These behaviours were quantified for 10-min, based on films of separate workers. Each worker was tested only once. We used dead workers as opponents to eliminate the sources of variation in behaviour of the tested ant workers, related to their responses to different behaviour of live ants introduced into their isolation arena (similar to Crosland, 1990; Scharf et al., 2011). The opponents were killed by freezing at  $-20^{\circ}\text{C}$  when all workers were taken out of the colony for the experiment and were kept under deep-freeze. They were singly defrosted 1 min before usage. One isolated worker per isolation period was used for two measurements of body reserves, based on photos taken under a stereomicroscope: the gaster width to head width ratio and the body mass to head width ratio. Head width is invariable in adult insects and is generally used as a measure of body size. Gaster width can vary with the amount of body reserves, the filling of the crop, and ovary development in queens. A previous study on the studied ant (*T. nylanderi*) has demonstrated a stronger correlation between gaster-to-head width and survival time under isolation than in groups (Modlmeier et al., 2013). Mass was measured using an analytical scale (accuracy of 0.01 mg). For both measurements, a larger value indicates more reserves relative to body size, similar to the body mass index. Finally, two workers per colony and isolation period were pooled for brain gene expression analyses, to obtain a sufficient amount of RNA for the analysis of gene expression and to reduce the between-worker variance within each colony. All tests were conducted between 09:30–15:30 h, with workers from the same colony being tested at the same time of day ( $\pm$  1 h). None of the workers died a natural death during the experiment, although several workers (<10) drowned in the honey plate and were removed from the analysis.

### 2.3 | Statistical analyses

We performed all the analyses with the software R (v. 4.0.2). Before analysis, the data were transformed into a long format with an explanatory variable “measurement” containing the eight measurements as levels and a response variable “value” containing the scaled values for each measurement. As the eight measurements related to behaviour and body reserves could be interrelated, we conducted a multivariate mixed-effect model to quantify the effect of isolation on those measurements. We tested for the effect of isolation duration, as a continuous variable, on the eight measurements by contrasting these two linear mixed-effect models, each built using the lmer() function of the lme4 package (by Dworkin & Bolker):

- Full model:  $\text{Imer}(\text{value} \sim \text{measurement} + \text{measurement}:\text{time} + (\text{measurement}-1|\text{colony}))$
- Reduced model:  $\text{Imer}(\text{value} \sim \text{measurement} + (\text{measurement}-1|\text{colony}))$

We compared the models with an approximate F test based on the Kenward-Roger approach, using the `KRmodcomp()` function from the `pbkrtest` R package. We plotted the intercept with the confidence intervals for the interaction term of each measurement with isolation duration, which revealed that some measurements had changed accordingly. We confirmed these multivariate analyses with independent models (Appendix S2).

## 2.4 | Differential gene expression

We flash-froze two workers per colony per isolation period in liquid nitrogen and transferred them to vials at  $-80^{\circ}\text{C}$ . The worker brains were dissected and placed together in Trizol. RNA was extracted using the RNeasy Mini Extraction kit (Qiagen) and was sent to Beijing Genomics Institute (BGI) for library construction and sequencing of 100 base pair (bp) paired-end reads using BGISEQ (for raw reads see NCBI, BioProject PRJNA608848). The pooled brains contained 0.0208–0.1201  $\mu\text{g}$  of RNA. The reads were quality- and adapter-trimmed (`TRIMMOMATIC v0.39`) and passed a quality check (`FASTQC v0.11.8`). We used a genome-guided assembly, and the paired forward and reverse reads were mapped against the genome of *T. nylanderii* (Jongepier et al., Unpublished) (`HISAT2 v2.1.0`). We assembled the RNA reads into potential transcripts (`STRINGTIE v1.3.6`) based on the mappings obtained against the genome. We extracted the transcript sequences (`GFFREAD v0.11.4`) and used transcripts of an open reading frame of at least 150 bp for later analyses. The assembly of the trimmed paired reads using `StringTie` resulted in 74,928 contigs, with a mean length of 3093 bp. The transcriptome filtered after ORF length and minimum read count comprised 41,467 contigs. We compared the transcripts to known invertebrate gene products (protein sequences downloaded from NCBI in April 2019, a database created using `makeblastdb`, `BLASTX v2.9.0`), using an e-value threshold of  $10^{-5}$ . We annotated our translated transcripts (`TRANSDCODER v5.5.0`, and `INTERPROSCAN v5.34-73.0`) for the gene ontology (GO) analysis. Later analyses were conducted in R (2013). We filtered for the transcripts, having at least 10 read counts in at least six samples. We used `DESeq2 (v1.24.0)` to identify differentially expressed genes. We used likelihood-ratio tests (LRT) to compare a full model ( $\sim\text{colony} + \text{bs}(\text{isolation time})$ ) to a reduced model ( $\sim\text{colony}$ ). This allowed us to identify genes affected by isolation time while controlling for colony effects. We used the `bs()` function (default parameters) from the R package `splines (3.6.0)` to also detect nonlinear changes in expression over isolation time. The transcripts of interest were checked for GO enrichment using `topGO (v2.36.0)` (Appendix S3). We used the `degPatterns` function (with parameter `minc = 3`) of the R package `DEGreport (v1.20.0)` to cluster differentially

expressed genes according to their expression patterns over isolation time (Appendix S4).

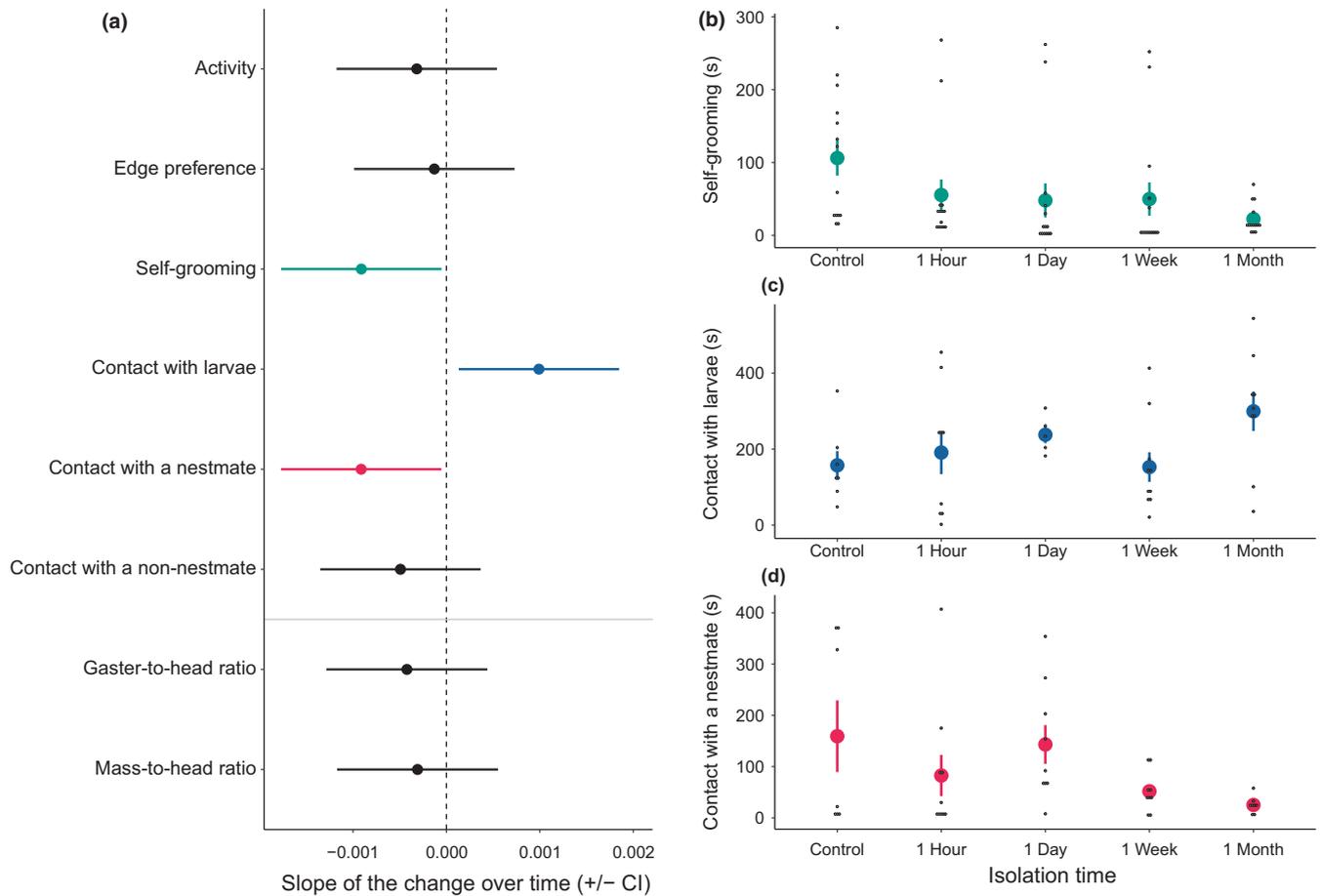
## 3 | RESULTS

### 3.1 | Changes in behaviour and body reserves

Our multivariate analysis revealed an interaction between isolation duration and the behavioural and morphological measurements ( $F_{8,438.3} = 2.13$ ,  $p = 0.03$ ). The slope of changes over isolation duration differed from zero for three of our measurements: self-grooming, contact with larvae, and contact with a nestmate, indicating that they had changed with social isolation (Figure 1a). With increasing isolation, workers exhibited shorter durations of self-grooming and contact with adult nestmates, but longer durations of interactions with larvae (Figure 1b–d). Activity, edge preference, and contact with non-nestmates did not shift with social isolation. Body reserve measurements also did not vary with the duration of isolation. Independent analyses confirmed these findings, while indicating that the effect of isolation on behaviour was moderate (Appendix S1). Colonies were kept for different periods in the laboratory (21–58 days) before the experiment commenced. Therefore, workers from the one month isolation treatment of the first experimental colonies and workers from the control/1 h/1 day treatment of the last experimental colonies were tested around the same time (Appendix S1). While isolation is confounded with time in the laboratory for each colony, the time varies strongly across colonies making it unlikely that maintenance time in the laboratory can explain our findings.

### 3.2 | Changes in brain gene expression

In total, 87 contigs were differentially expressed with isolation duration (false discovery rate  $< 0.05$ ). According to the `BLASTx` search against the invertebrate database (August 2019), the products of several differentially expressed genes were related to immune system functioning and stress. We summarized the functions of interesting candidate genes (Table 1). A gene encoding adenosine deaminase 2-like decreased with isolation duration (Figure 2a). This gene is involved in immune response, the differentiation of monocytes, inflammatory and injury responses, phagocytosis and melanization/encapsulation of foreign bodies (Novakova & Dolezal, 2011; Zavalov et al., 2010). Aromatic-L-amino-acid decarboxylase-like plays a role in the production of dopamine and serotonin (Han et al., 2010; Vavricka et al., 2014). We found an increase in its production after a single day of isolation, followed by a decrease back to its original levels (Figure 2b). The expression of four Cytochrome P450 genes either decreased after a single day of isolation, or increased and then decreased with isolation duration (Figure 2c,d). These Cytochrome P450 genes are involved in immune defence and help to remove toxic materials such as



**FIGURE 1** (a) Multivariate analysis examining whether isolation duration affects the phenotypic measurements. Isolation affected only self-grooming, duration of contact with larvae, and duration of contact with a nestmate. CI stands for the 95% confidence intervals. (b) The negative effect of isolation duration on self-grooming, (c) the positive effect of isolation duration on the duration of contact with larvae; and (d) the negative effect of isolation duration on the duration of contact with a nestmate. In b–d, Mean  $\pm$  1 SE are presented as well as all data points (black dots).

insecticides (Yocum et al., 2018; Zhang et al., 2016). The expression of a gene involved in thermal tolerance, and probably also in coping with other stressors (heat shock 70 kDa protein cognate 5; Yuan et al., 2017) decreased with isolation, following an increase after one day of isolation (Figure 2e). The expression of a gene that encodes a protein catabolizing juvenile hormone (Juvenile hormone epoxide hydrolase 1-like; Toxopeus et al., 2016; Allen et al., 2018) decreased with the isolation period (Figure 2f). This probably results in higher levels of juvenile hormones with isolation, unless juvenile hormone levels are elevated in parallel with isolation, for which we did not find any evidence. The apparent link between the possible maintenance of higher levels of juvenile hormone and longer durations of brood care by isolated workers may also play a role in the ontogeny of ant behaviour, and in particular the transition from intranidal to extranidal activities in this and other ant species. The transition from intranidal to extranidal activities is accompanied by an increase of juvenile hormone levels (e.g., Jaycox, 1976). Our findings – social isolation leading to an increase in contacts with brood and to a decrease in the expression of the gene catabolizing juvenile hormone (which should lead to

increased levels of that hormone) – are somewhat contradictory. However, it is also known that extranidal workers respond readily to brood found outside the nest (Lenoir, 1981). The expression of a gene involved in TOR signalling (protein charybde-like; Gotoh et al., 2018) decreased with isolation (Figure 2g). Interference with this pathway is associated with stress and shortened longevity (Amdam, 2011). A gene involved in controlling the inhibition of the activity of the insect visceral muscles (Myosuppressin; Egerod et al., 2003; Lee et al., 2015) increased with isolation (Figure 2h), potentially interfering with food intake and digestion.

To examine whether time in the laboratory or handling could explain the transcriptomic changes we report, we conducted two additional comparisons. We contrasted gene expression immediately following isolation with one day isolation and 1 h isolation with one month isolation. Five out of eight candidate genes from our gene expression analysis were already differently expressed after one day of isolation indicating that the detected differences are triggered by isolation time. Similarly, we found five of eight candidates to be differently expressed between 1 h and one month isolation, revealing that handling alone, i.e., transfer into

**TABLE 1** Candidate genes changing their expression with the isolation of ant workers. I, D, and ID stand for an increase, a decrease, or an increase followed by a decrease in expression with isolation duration over time and the false discovery rate (FDR), or type 1 error value is equivalent to the *p*-value

Blast annotation (species)	I/D	Log-fold ch., FDR	Function (based on previous literature)	References
Adenosine deaminase 2-like (Tc)	D	1.54, $5.8 \times 10^{-8}$	Immunity: differentiation of macrophages inflammatory responses	Zavialov et al. (2010), Novakova & Dolezal (2011)
Aromatic-L-amino-acid decarboxylase-like (Tc)	ID	0.04, $1.2 \times 10^{-6}$	Serotonin/dopamine production, neurotransmission, insect immune response	Han et al. (2010), Vavricka et al. (2014)
Juvenile hormone epoxide hydrolase 1-like (Tc)	D	1.22, 0.0001	Catalyses juvenile hormone (JH) hydrolysis; JH is involved in caste determination, mediation of behaviour.	Toxopeus et al. (2016), Allen et al. (2018)
Myosuppressin (Tc)	I	1.22, 0.0002	Decapeptide inhibiting insect visceral muscles, e.g., impedes food intake.	Egerod et al. (2003), Lee et al. (2015)
Cytochrome P450 6a2-like (Tc)	ID	3.80, 0.0005	Insecticide breakdown, insect hormone metabolism, possibly involved in the ant immune response	Brun et al. (1996), Ratzka et al. (2011)
Cytochrome P450 4C1-like isoform X1 (Tc)	D	4.31, 0.0006	Insect hormone metabolism, insecticide breakdown, also in ants	Zhang et al. (2016)
Cytochrome P450 6a14 (Tc)	ID	0.34, 0.003	Insect hormone metabolism, insecticide breakdown, seasonal expression in bees	Yocum et al. (2018)
Cytochrome P450 4C1-like (Tc)	D	1.66, 0.004	Insect hormone metabolism, insecticide breakdown, also in ants	Yang et al. (2013), Zhang et al. (2016)
Predicted heat shock 70 kDa protein cognate 5 (Wa)	ID	0.35, 0.005	Response to stress, heat stress, upregulated under viral stress	Yuan et al. (2017)
Something about silencing protein 10 (Tc)	ID	0.16, 0.027	Roles in: gene silencing, the structure of silenced chromatin, gene regulation during development, RNA binding	Peters et al. (2003)
Predicted protein charybde-like (Cc)	D	-0.24, 0.034	Apoptotic process, negative regulation of signal transduction, regulation of TOR signalling, expressed in the ant accessory glands	Gotoh et al. (2018)

Tc, *Temnothorax curvispinosus*; Wa, *Wasmannia auropunctata*; Cc, *Cyphomyrmex costatus*. The function of the up- or downregulated genes is suggested based on functional annotation and existing literature.

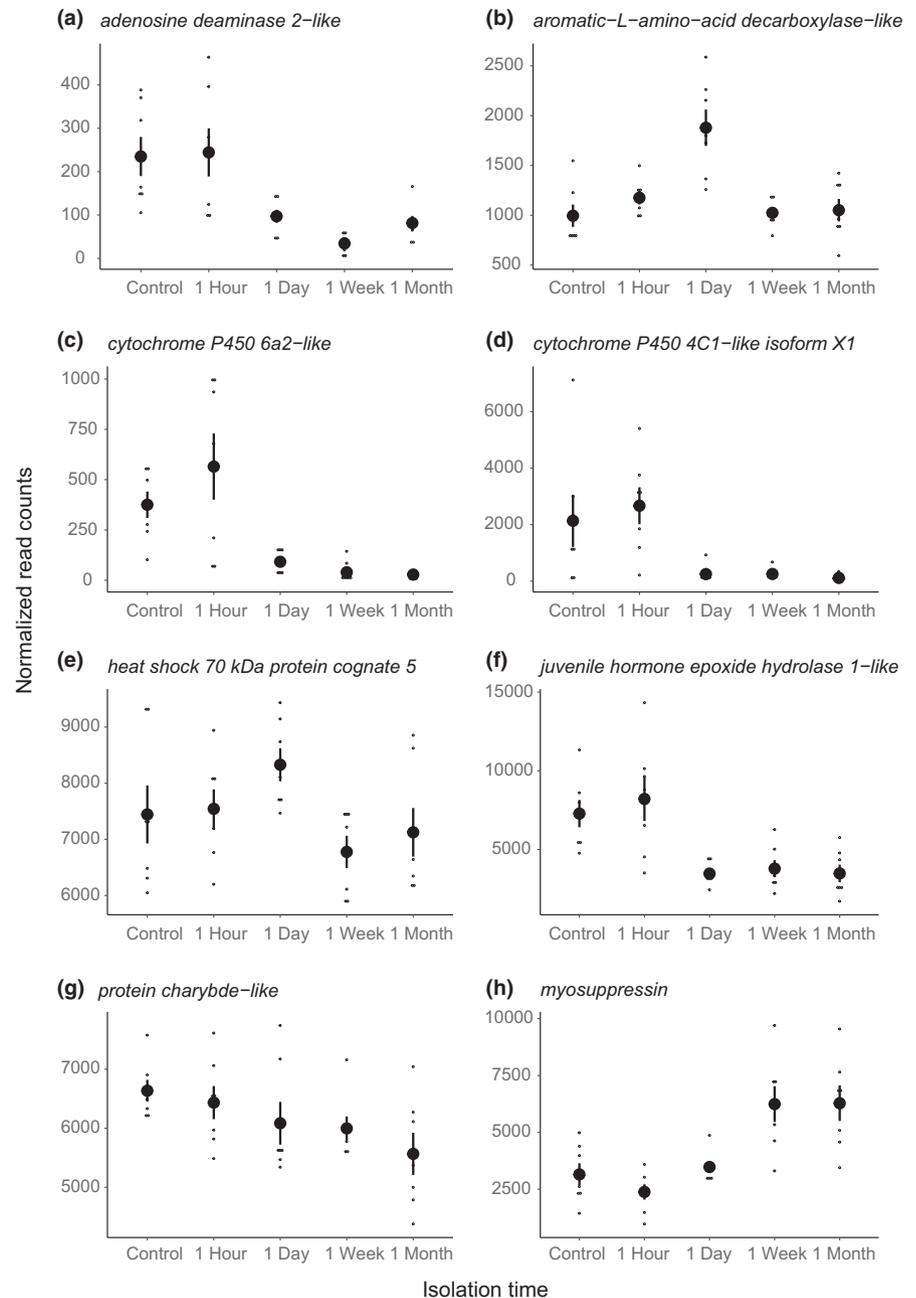
an arena, which was identical in both treatments, cannot explain the described changes (Appendix S2). These additional contrasts therefore confirm that isolation duration is indeed responsible for the reported transcriptomic shifts.

## 4 | DISCUSSION

Social insects are surrounded by nestmates. Except for rare occasions, such as during social withdrawal when workers are injured/infected, queens found a colony, or individually foraging workers, social interactions make up most of their daily life. Isolation over longer periods should therefore lead to profound changes in behaviour and physiology. We found clear changes in brain gene expression and alterations in three important behaviours, although the behavioural shifts were not highly pronounced. As *T. nylander* workers live in small societies and mostly forage on their own, they are possibly more resilient and can withstand periods

of solitude. We found that the self-grooming sanitary behaviour decreased with social isolation. Following isolation, ant workers also contacted their nestmates slightly less when re-encountering their worker sisters after hours or days. With increasing isolation time, workers moderately increased their contacts with larvae when given the opportunity. Other behaviours, such as activity or the response to non-nestmates and their body reserves did not change following social isolation. Our transcriptome analyses revealed strong changes in the expression of over 80 genes in the brains of workers following isolation. Isolated workers downregulated immune and stress-related genes, indicating that isolation causes immune suppression in social insects. This finding parallels the decrease in self-grooming behaviours detected with ongoing isolation, making workers that suffer from social isolation more susceptible to infection. Many downregulated genes have functionalities, such as chemical stress resistance, suggesting that social isolation may limit the ability of ants to cope with these stressors too.

**FIGURE 2** Candidate gene expression changes as a function of isolation period. (a) Adenosine deaminase 2-like, (b) aromatic-L-amino-acid decarboxylase-like, (c) cytochrome P450 6a2-like, (d) cytochrome P450 4C1-like isoform X1, (e) heat shock 70 kDa protein cognate 5, (f) juvenile hormone epoxide hydrolase 1-like, (g) protein charybde-like and (h) myosuppressin. False discovery rate (FDR) values are provided. Mean  $\pm$  1 SE are presented as well as all data points (black dots).



#### 4.1 | Behavioural and body reserve changes with isolation

With increasing isolation duration, workers displayed moderately less interest in contacting nestmates, when a nestmate worker was reintroduced into their arena. Similar findings of lower antennation rates following social isolation were reported in the ant *Camponotus acvapimensis* (Cybulska et al., 2000). The reduced interest in nestmates following isolation resembles that observed in small mammals, which often exhibit a reduction in social interactions, linked to an increase in anxiety-like behaviours following social isolation (e.g., Lukkes et al., 2009). A possible, social insect-specific cause of the social contact reduction could be that of a gradual divergence in the cuticular hydrocarbon profile of workers isolated from their

nestmates. Such a shift has been shown in the ant *Camponotus fellah*, where it led to increased aggression between nestmates following isolation (Ichinose & Lenoir, 2009; Lenoir et al., 2001). Our focal ants' responses towards non-nestmates did not shift with isolation and were generally weak. We never observed overt aggression probably due to the generally low aggression levels in our focal species (Beros et al., 2017), or because encounters happened outside of the nest, which is generally defended (Scharf et al., 2012). Studies on insects have demonstrated mixed patterns regarding changes in aggression with isolation (Boulay & Lenoir, 2001; Breed, 1983; Korczyńska et al., 2005; Liu et al., 2011; Stevenson & Rillich, 2013).

Self-grooming is an effective sanitary behaviour used by ants to clean their cuticle from fungal or bacterial pathogens (Tragust et al., 2013). It is also involved in other functions, such as homogeneization

of the colony odour by spreading over the body the contents of post-pharyngeal glands exchanged between ant workers via trophallaxis and allogrooming (Soroker et al., 1995). Exposure to a pathogenic fungus was shown to cause an increase in self-grooming but a decrease in allo-grooming in the ant *Lasius neglectus* (Theis et al., 2015). Here, we found that workers exposed to social isolation showed a reduction in self-grooming, which, in addition to the downregulation of immune genes, is expected to make isolated workers more susceptible to pathogens. Contact with re-introduced larvae moderately increased following prolonged isolation, which could be partially explained by the finding from earlier work on ants, of a negative association between the intensity of larval care and self-grooming (Westhus et al., 2014). The evidence from social mammals is to the contrary: isolation and the deprivation of maternal care in juvenile rats lead to their later reduction in care of their own offspring (Lovic et al., 2001).

Social isolation can affect activity patterns of animals. In the ant *Camponotus fellah* isolated workers become more active, but only on the first day of isolation (Koto et al., 2015). Rodents exhibit diverse activity changes with social isolation (Grippio et al., 2008; Silva-Gomez et al., 2003; Weiss et al., 2004). *Temnothorax* workers become typically more active with age (Kohlmeier et al., 2017), but our experiments found no change in activity with social isolation over time. Caste too can have a strong influence on the response to social isolation, with nurses being more ready to engage in social contact, while foragers are more interested in their immediate abiotic environment (Mazurkiewicz et al., 2016; Wagner-Ziemka et al., 2008). Ant nurses and foragers may also differ with respect to their mortality in conditions of social isolation (Boulay et al., 1999). The tendency to move along the test arena's edges reflects either the risk that the tested animal perceives or its attempt to escape a closed area. Although this tendency has been shown to change with isolation duration in rats and zebrafish (Djordjevic et al., 2012; Shams et al., 2015), we did not observe any such change here.

Body reserves did not change with isolation. This deviates from previous findings for the same species, which demonstrated lower body reserves in isolated than in grouped workers (Modlmeier et al., 2013). The difference between the two studies lies in the fact that our workers were allowed to feed, whereas in the study of Modlmeier et al., (2013) the workers were starved. Our finding also contrasts those of other studies on isolated ants that have reported digestion problems (Howard & Tschinkel, 1981; Koto et al., 2015), which should lead to reduced body reserves. It is possible that other nutritional deficits exist but are not detectable using this rough estimator, and more accurate biochemical methods are required to detect an effect of social isolation.

Three confounding factors that we were unable to separate from isolation duration might have affected our results. First, the period that each colony was maintained in the laboratory is confounded with isolation duration. During this period, other behavioural changes could have taken place, such as a transition from nursing to foraging in some workers. Foragers could be more interested in returning brood to the nest (Lenoir, 1981), which fits the observed

behavioural change of increased interactions with brood following isolation. Furthermore, the transition from intranidal to extranidal activities might have been accelerated in isolated ants by the possibly higher levels of juvenile hormone in the brain of isolated workers (e.g., Jaycox, 1976). However, given the lifespan of *Temnothorax* workers of several years and the once per annum production of new workers, behavioural progression is unlikely to explain the observed behavioural changes given the timeframe of our study. Second, isolation is confounded with changes in nest structure (to a Petri dish), which may also affect behaviour. However, this should have only affected the ants in the control, as thereafter the nest structure remained unmodified. Third, isolation was confounded with protein-free nutrition and the absence of brood. In social Hymenoptera, larvae are often responsible for breaking down the protein into amino acids and provisioning the workers (Schultner et al., 2017). Thus, some behavioural changes, such as higher attendance to larvae upon reunification could result from a reduction in the proteins available to the isolated workers.

## 4.2 | Brain gene expression changes with isolation

We demonstrate that the expression of stress or immune system-associated genes was linked to isolation duration. The downregulation of genes involved in the immune response should impede the ability of ant workers to confront a challenge to the immune system. Our findings thus fit the results on other group-living insects and mammals, indicating that the deterioration of the immune system following isolation is widespread across social animals: earwigs and termites isolated after fungal infection died faster than those also infected but kept in groups (Hughes et al., 2002; Rosengaus et al., 1998), and isolated mammals have been shown to have a higher risk of developing cancer (Wu et al., 2000). The mechanisms behind a suppressed immune system include lower levels of hemocyte cells in the hemolymph of isolated moths (Pavlushin et al., 2019); and lower activity of natural killer cells and lymphocytes in lonely/isolated humans and mice than in nonlonely/grouped ones (Cruces et al., 2014; Kiecolt-Glaser et al., 1984).

Genes related to the neutralization of toxins are downregulated as well with isolation in our ants. These genes are involved in insecticide resistance in fruitflies, whiteflies, and fire ants (Brun et al., 1996; Yang et al., 2013; Zhang et al., 2016). Consequently, isolation should make insects more vulnerable to insecticides and toxins. Isolation in small mammals leads to elevated oxidative stress in the brain, because more reactive substances are produced and the activity of antioxidant enzymes is lower (Huong et al., 2005; Shao et al., 2015), suggesting that isolated small mammals cope less well with toxic materials. Similarly, social isolation increases the expression of heat-shock proteins in mammals, and such upregulation may help to protect the brain against reactive oxygen species (Zlatković, Bernardi, et al., 2014; Zlatković, Todorović, et al., 2014).

Some genes were initially expressed more intensely with short isolation duration and then expressed less when isolation was

prolonged. We found such a pattern for the gene encoding Aromatic-L-amino-acid decarboxylase-like, involved in the production of dopamine and serotonin (Han et al., 2010; Vavricka et al., 2014). These neurotransmitters are linked to anxiety-related behaviours in mammals (Brandão et al., 2015; Marcinkiewicz et al., 2016), and an increase in anxiety is a typical response of mammals to social isolation (Bibancos et al., 2007; Pan et al., 2009). We are not suggesting that animals lacking social contact are more anxious. Rather, we are reporting on a change following isolation in expression of a gene involved in molecular pathways associated with anxiety in mammals. In insects, serotonin is involved in the mediation of behaviour and physiology, such as feeding, sleeping, and learning (Vleugels et al., 2015). Serotonin and dopamine also play a role in the mediation of aggressive behaviour and other social activities in ants (Kostowski & Tarchalska, 1972; Kostowski et al., 1975; Szczuka et al., 2013). In contrast to the observed change in this gene's expression, which reached its peak after a single day of isolation, we did not detect any behavioural changes. Koto et al., (2015) observed an increase in the activity of isolated workers, but only on the first day of isolation, which appears to correspond well to our finding: insect activity is often positively correlated with serotonin levels (Kamyshev et al., 1983; Kloppenburg et al., 1999).

The decrease in expression of a gene responsible for catabolizing juvenile hormone with isolation duration could lead to higher circulating levels of juvenile hormone. In agreement, honeybees and locusts raised/maintained in isolation demonstrated higher levels of juvenile hormone (Breed, 1983; Huang & Robinson, 1992; Injeyan & Tobe, 1981). A gene encoding Myosuppressin, a protein involved in controlling the inhibition of the activity of the insect visceral muscles, and which could interfere with food intake and digestion, is upregulated with isolation. The upregulation of this gene may indicate impaired digestion in isolated workers. Isolated workers were shown to have retained food in their foregut (Koto et al., 2015), and Myosuppressin can inhibit foregut activity (Aguilar et al., 2004). This upregulation may explain why the body reserve measurements did not differ between isolated and control ants: food was retained in the gut but was not digested, leading to no observed change in morphology.

## 5 | CONCLUSIONS

We have demonstrated that pronounced changes in gene expression, correlated with social isolation, take place in *T. nylanderi* workers. These changes may have adverse effects on the ants as they indicate suppression of the immune system, lower resistance to other stressors, interference with food intake and digestion, and a possible increase in circulating juvenile hormone. Such changes might contribute to a shortened lifespan in isolated workers, as detected for this and other ants. We found moderate changes in social interactions following isolation: ant workers were less inclined to contact their adult nestmates, while increasing the durations of contacts with brood. The reduction in self-grooming with ongoing isolation should reduce the removal of pathogens from the cuticle and, in

combination with downregulation of immune genes, might make isolated workers more prone to infection. Future studies should examine how behavioural and physiological immunity variables interact and analyse the behavioural responses to isolation in more depth. Such studies should also include physiological and neurobiological factors. Only such multifaceted examinations can help us to reach a firm conclusion regarding the comprehensive effects of isolation in social species.

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## AUTHOR CONTRIBUTIONS

I.S., S.F., and R.L. designed the study and interpreted its results. S.F. and I.S. collected the ant colonies. I.S., A.L.H., and M.K. conducted the experiment. R.L., E.J. and I.S. analysed the behavioural data. M.S., R.L. and I.S. analysed the transcriptomics data. E.J. contributed genomic data of the studied species. I.S. and S.F. wrote the manuscript. The study was conducted in the laboratory of S.F. All authors contributed to writing and approved the final manuscript.

## DATA AVAILABILITY STATEMENT

The behavioural data are available as Supporting Information. The transcriptomics raw data have been uploaded to: [www.ncbi.nlm.nih.gov/bioproject/608848](http://www.ncbi.nlm.nih.gov/bioproject/608848), and will be available after publication.

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## REFERENCES

- Aguilar, R., Maestro, J. L., Vilaplana, L., Chiva, C., Andreu, D., & Bellés, X. (2004). Identification of leucomyosuppressin in the German cockroach, *Blattella germanica*, as an inhibitor of food intake. *Regulatory Peptides*, *119*, 105–112.
- Allen, M., Rhoades, J., Sparks, M., & Grodowitz, M. (2018). Differential gene expression in red imported fire ant (*Solenopsis invicta*) (Hymenoptera: Formicidae) larval and pupal stages. *Insects*, *9*, 185.
- Amdam, G. V. (2011). Social context, stress, and plasticity of aging. *Aging Cell*, *10*, 18–27.
- 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.
- Bibancos, T., Jardim, D. L., Aneas, I., & Chiavegatto, S. (2007). Social isolation and expression of serotonergic neurotransmission-related

- genes in several brain areas of male mice. *Genes, Brain & Behavior*, 6, 529–539.
- Boulay, R., & Lenoir, A. (2001). Social isolation of mature workers affects nestmate recognition in the ant *Camponotus fellah*. *Behavioural Processes*, 55, 67–73.
- Boulay, R., Quagebeur, M., Godzinska, E. J., & Lenoir, A. (1999). Social isolation in ants: evidence of its impact on survivorship and behavior in *Camponotus fellah* (Hymenoptera: Formicidae). *Sociobiology*, 33, 111–124.
- Bowen, M. T., Kevin, R. C., May, M., Staples, L. G., Hunt, G. E., & McGregor, I. S. (2013). Defensive aggregation (huddling) in *Rattus norvegicus* toward predator odor: individual differences, social buffering effects and neural correlates. *PLoS One*, 8, e68483.
- Brandão, M. L., de Oliveira, A. R., Muthuraju, S., Colombo, A. C., Saito, V. M., & Talbot, T. (2015). Dual role of dopamine D2-like receptors in the mediation of conditioned and unconditioned fear. *FEBS Letters*, 589, 3433–3437.
- Breed, M. D. (1983). Correlations between aggressiveness and corpora allata volume, social isolation, age and dietary protein in worker honeybees. *Insectes Sociaux*, 30, 482–495.
- Brennan, M., Ali, B., Robinson, S. M., Mulkerrin, E. C., & O’Keeffe, S. T. (2019). Indications and outcomes of home visits by geriatricians. *European Geriatric Medicine*, 10, 511–516.
- Brun, A., Cuany, A., Le Mouel, T., Berge, J., & Amichot, M. (1996). Inducibility of the *Drosophila melanogaster* cytochrome P450 gene, CYP6A2, by phenobarbital in insecticide susceptible or resistant strains. *Insect Biochemistry and Molecular Biology*, 26, 697–703.
- Cacioppo, J. T., Cacioppo, S., Capitanio, J. P., & Cole, S. W. (2015). The neuroendocrinology of social isolation. *Annual Review of Psychology*, 66, 733–767.
- Cacioppo, J. T., Hawkley, L. C., Norman, G. J., & Berntson, G. G. (2011). Social isolation. *Annals of the New York Academy of Sciences*, 1231, 17–22.
- Champalbert, A., & Lachaud, J. P. (1990). Existence of a sensitive period during the ontogenesis of social behaviour in a primitive ant. *Animal Behaviour*, 39, 850–859.
- Cole, S. W., Hawkley, L. C., Arevalo, J. M., Sung, C. Y., Rose, R. M., & Cacioppo, J. T. (2007). Social regulation of gene expression in human leukocytes. *Genome Biology*, 8, R189.
- Courtin, E., & Knapp, M. (2017). Social isolation, loneliness and health in old age: a scoping review. *Health & Social Care in the Community*, 25, 799–812.
- Crosland, M. W. (1990). Variation in ant aggression and kin discrimination ability within and between colonies. *Journal of Insect Behaviour*, 3, 359–379.
- Cruces, J., Venero, C., Pereda-Pérez, I., & De la Fuente, M. (2014). A higher anxiety state in old rats after social isolation is associated to an impairment of the immune response. *Journal of Neuroimmunology*, 277, 18–25.
- Cybulska, A., Godzinska, E. J., & Wagner-Ziemka, A. (2000). Behaviour of dyads of ants reunited after social deprivation. *Biological Bulletin of Poznan*, 37, 119–127.
- Denmark, A., Tien, D., Wong, K., Chung, A., Cachat, J., Goodspeed, J., Grimes, C., Elegante, M., Suci, C., Elkhayat, S., Bartels, B., Jackson, A., Rosenberg, M., Chung, K. M., Badani, H., Kadri, F., Roy, S., Tan, J., Gaikwad, S., ... Kalueff, A. V. (2010). The effects of chronic social defeat stress on mouse self-grooming behavior and its patterning. *Behavioural Brain Research*, 208, 553–559.
- Djordjevic, J., Djordjevic, A., Adzic, M., & Radojic, M. B. (2012). Effects of chronic social isolation on Wistar rat behavior and brain plasticity markers. *Neuropsychobiology*, 66, 112–119.
- Egerod, K., Reynisson, E., Hauser, F., Cazzamali, G., Williamson, M., & Grimmelikhuijzen, C. J. (2003). Molecular cloning and functional expression of the first two specific insect myosuppressin receptors. *Proceedings of the National Academy of Sciences USA*, 100, 9808–9813.
- Fone, K. C., & Porkess, M. V. (2008). Behavioural and neurochemical effects of post-weaning social isolation in rodents – relevance to developmental neuropsychiatric disorders. *Neuroscience & Biobehavioral Reviews*, 32, 1087–1102.
- Gotoh, A., Shigenobu, S., Yamaguchi, K., Kobayashi, S., Ito, F., & Tsuji, K. (2018). Transcriptome characterization of male accessory glands in ants to identify molecules involved in their reproductive success. *Insect Molecular Biology*, 27, 212–220.
- Grippe, A. J., Wu, K. D., Hassan, I., & Carter, C. S. (2008). Social isolation in prairie voles induces behaviors relevant to negative affect: toward the development of a rodent model focused on co-occurring depression and anxiety. *Depression & Anxiety*, 25, E17–E26.
- Han, Q., Ding, H., Robinson, H., Christensen, B. M., & Li, J. (2010). Crystal structure and substrate specificity of *Drosophila* 3, 4-dihydroxyphenylalanine decarboxylase. *PLoS One*, 5, e8826.
- Hawkley, L. C., & Capitanio, J. P. (2015). Perceived social isolation, evolutionary fitness and health outcomes: a lifespan approach. *Philosophical Transactions of the Royal Society B*, 370, 20140114.
- Hawkley, L. C., Cole, S. W., Capitanio, J. P., Norman, G. J., & Cacioppo, J. T. (2012). Effects of social isolation on glucocorticoid regulation in social mammals. *Hormones & Behavior*, 62, 314–323.
- Howard, D. F., & Tschinkel, W. R. (1981). Internal distribution of liquid foods in isolated workers of the fire ant, *Solenopsis invicta*. *Journal of Insect Physiology*, 27, 67–74.
- Huang, Z. Y., & Robinson, G. E. (1992). Honeybee colony integration: worker-worker interactions mediate hormonally regulated plasticity in division of labor. *Proceedings of the National Academy of Sciences*, 89, 11726–11729.
- Hughes, W. 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 B*, 269, 1811–1819.
- Huong, N. T. T., Murakami, Y., Tohda, M., Watanabe, H., & Matsumoto, K. (2005). Social isolation stress-induced oxidative damage in mouse brain and its modulation by majonoside-R2, a Vietnamese ginseng saponin. *Biological and Pharmaceutical Bulletin*, 28, 1389–1393.
- Ichinose, K., & Lenoir, A. (2009). Ontogeny of hydrocarbon profiles in the ant *Aphaenogaster senilis* and effects of social isolation. *Comptes Rendus Biologies*, 332, 697–703.
- Injeyan, H. S., & Tobe, S. S. (1981). Phase polymorphism in *Schistocerca gregaria*: assessment of juvenile hormone synthesis in relation to vitellogenesis. *Journal of Insect Physiology*, 27, 203–210.
- Jaycox, E. R. (1976). Behavioral changes in worker honey bees (*Apis mellifera* L.) after injection with synthetic juvenile hormone (Hymenoptera: Apidae). *Journal of the Kansas Entomological Society*, 49, 165–170.
- Kamyshev, N. G., Smirnova, G. P., Savvateeva, E. V., Medvedeva, A. V., & Ponomarenko, V. V. (1983). The influence of serotonin and p-chlorophenylalanine on locomotor activity of *Drosophila melanogaster*. *Pharmacology Biochemistry and Behavior*, 18, 677–681.
- Kanitz, E., Tuchscherer, M., Puppe, B., Tuchscherer, A., & Stabenow, B. (2004). Consequences of repeated early isolation in domestic piglets (*Sus scrofa*) on their behavioural, neuroendocrine, and immunological responses. *Brain, Behavior, & Immunity*, 18, 35–45.
- Kiecolt-Glaser, J. K., Ricker, D., George, J., Messick, G., Speicher, C. E., Garner, W., & Glaser, R. (1984). Urinary cortisol levels, cellular immunocompetency, and loneliness in psychiatric inpatients. *Psychosomatic Medicine*, 46, 15–23.
- Kloppenborg, P., Ferns, D., & Mercer, A. R. (1999). Serotonin enhances central olfactory neuron responses to female sex pheromone in the male sphinx moth *Manduca sexta*. *Journal of Neuroscience*, 19, 8172–8181.
- Kohlmeier, P., Feldmeyer, B., & Foitzik, S. (2018). Vitellogenin-like A-associated shifts in social cue responsiveness regulate behavioral task specialization in an ant. *PLoS Biology*, 16, e2005747.
- 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. *Science of Nature*, 104, 34.
- Korczyńska, J., Szczuka, A., Kieruzel, M., Majczynski, H., Khvorostova, N., & Godzińska, E. J. (2005). Effects of the biogenic amines, dopamine, tyramine and octopamine on the behavior of carpenter ant workers [*Camponotus herculeanus* (Hymenoptera: Formicidae)] during nestmate reunion tests carried out after a period of social isolation. *Sociobiology*, 45, 409–447.
- Kostowski, W., & Tarchalska, B. (1972). The effects of some drugs affecting brain 5-HT on the aggressive behaviour and spontaneous electrical activity of the central nervous system of the ant, *Formica rufa*. *Brain Research*, 38, 143–149.
- Kostowski, W., Tarchalska, B., & Wańchowicz, B. (1975). Brain catecholamines, spontaneous bioelectrical activity and aggressive behavior in ants (*Formica rufa*). *Pharmacology Biochemistry & Behavior*, 3, 337–342.
- Koto, A., Mersch, D., Hollis, B., & Keller, L. (2015). Social isolation causes mortality by disrupting energy homeostasis in ants. *Behavioral Ecology & Sociobiology*, 69, 583–591.
- Kramer, B. H., Scharf, I., & Foitzik, S. (2014). The role of per-capita productivity in the evolution of small colony sizes in ants. *Behavioral Ecology & Sociobiology*, 68, 41–53.
- Kumari, A., Singh, P., Baghel, M. S., & Thakur, M. K. (2016). Social isolation mediated anxiety like behavior is associated with enhanced expression and regulation of BDNF in the female mouse brain. *Physiology & Behaviour*, 158, 34–42.
- Lee, D., James, T. E., & Lange, A. B. (2015). Identification, characterization and expression of a receptor for the unusual myosuppressin in the blood-feeding bug, *Rhodnius prolixus*. *Insect Molecular Biology*, 24, 129–137.
- Lenoir, A. (1981). Brood retrieving in the ant, *Lasius niger* L. *Sociobiology*, 6, 3–78.
- Lenoir, A., Cuisset, D., & Hefetz, A. (2001). Effects of social isolation on hydrocarbon pattern and nestmate recognition in the ant *Aphaenogaster senilis* (Hymenoptera, Formicidae). *Insectes Sociaux*, 48, 101–109.
- Liang, Z. S., Nguyen, T., Mattila, H. R., Rodriguez-Zas, S. L., Seeley, T. D., & Robinson, G. E. (2012). Molecular determinants of scouting behavior in honeybees. *Science*, 335, 1225–1228.
- Lihoreau, M., Brepson, L., & Rivault, C. (2009). The weight of the clan: even in insects, social isolation can induce a behavioural syndrome. *Behavioural Processes*, 82, 81–84.
- Liu, W., Liang, X., Gong, J., Yang, Z., Zhang, Y. H., Zhang, J. X., & Rao, Y. (2011). Social regulation of aggression by pheromonal activation of Or65a olfactory neurons in *Drosophila*. *Nature Neuroscience*, 14, 896–902.
- Lovic, V., Gonzalez, A., & Fleming, A. S. (2001). Maternally separated rats show deficits in maternal care in adulthood. *Developmental Psychobiology*, 39, 19–33.
- Lukkes, J. L., Mokin, M. V., Scholl, J. L., & Forster, G. L. (2009). Adult rats exposed to early-life social isolation exhibit increased anxiety and conditioned fear behavior, and altered hormonal stress responses. *Hormones & Behavior*, 55, 248–256.
- Marcinkiewicz, C. A., Mazzone, C. M., D'Agostino, G., Halladay, L. R., Hardaway, J. A., DiBerto, J. F., Navarro, M., Burnham, N., Cristiano, C., Dorrier, C. E., Tipton, G. J., Ramakrishnan, C., Kozicz, T., Deisseroth, K., Thiele, T. E., McElligott, Z. A., Holmes, A., Heisler, L. K., & Kash, T. L. (2016). Serotonin engages an anxiety and fear-promoting circuit in the extended amygdala. *Nature*, 537, 97–101.
- Mazurkiewicz, P. J., Wagner-Ziemka, A., Mirecka, A., & Godzińska, E. J. (2016). Behaviour of intranidal and extranidal major workers of the African carpenter ant *Camponotus maculatus* Fabricius (Hymenoptera: Formicidae) during dyadic nestmate reunion tests. *African Entomology*, 24, 307–320.
- Modlmeier, A. P., Foitzik, S., & Scharf, I. (2013). Starvation endurance in the ant *Temnothorax nylanderii* depends on group size, body size and access to larvae. *Physiological Entomology*, 38, 89–94.
- Nicholson, N. R. (2012). A review of social isolation: an important but under-assessed condition in older adults. *Journal of Primary Prevention*, 33, 137–152.
- Novakova, M., & Dolezal, T. (2011). Expression of *Drosophila* adenosine deaminase in immune cells during inflammatory response. *PLoS One*, 6, e17741.
- Pan, Y., Liu, Y., Young, K. A., Zhang, Z., & Wang, Z. (2009). Post-weaning social isolation alters anxiety-related behavior and neurochemical gene expression in the brain of male prairie voles. *Neuroscience Letters*, 454, 67–71.
- Pantel, M., Rehkopf, D., Jutte, D., Syme, S. L., Balmes, J., & Adler, N. (2013). Social isolation: a predictor of mortality comparable to traditional clinical risk factors. *American Journal of Public Health*, 103, 2056–2062.
- Pavlushin, S. V., Belousova, I. A., Chertkova, E. A., Kryukova, N. A., Glupov, V. V., & Martemyanov, V. V. (2019). The effect of population density of *Lymantria dispar* (Lepidoptera: Erebididae) on its fitness, physiology and activation of the covert nucleopolyhedrovirus. *European Journal of Entomology*, 116, 85–91.
- Peters, N. T., Rohrbach, J. A., Zalewski, B. A., Byrket, C. M., & Vaughn, J. C. (2003). RNA editing and regulation of *Drosophila* 4f-rnp expression by sas-10 antisense read through mRNA transcripts. *RNA*, 9, 698–710.
- Ratzka, C., Liang, C., Dandekar, T., Gross, R., & Feldhaar, H. (2011). Immune response of the ant *Camponotus floridanus* against pathogens and its obligate mutualistic endosymbiont. *Insect Biochemistry and Molecular Biology*, 41, 529–536.
- Rosengaus, R. B., Maxmen, A. B., Coates, L. E., & Traniello, J. F. (1998). Disease resistance: a benefit of sociality in the dampwood termite *Zootermopsis angusticollis* (Isoptera: Termopsidae). *Behavioral Ecology Sociobiology*, 44, 125–134.
- Scharf, I., Modlmeier, A. P., Fries, S., Tirard, C., & Foitzik, S. (2012). Characterizing the collective personality of ant societies: aggressive colonies do not abandon their home. *PLoS One*, 7, e33314.
- Scharf, I., Pamminer, T., & Foitzik, S. (2011). Differential response of ant colonies to intruders: attack strategies correlate with potential threat. *Ethology*, 117, 731–739.
- Schultner, E., Oettler, J., & Helanterä, H. (2017). The role of brood in eusocial Hymenoptera. *Quarterly Review of Biology*, 92, 39–78.
- Seid, M. A., & Junge, E. (2016). Social isolation and brain development in the ant *Camponotus floridanus*. *Science of Nature*, 103, 42.
- Shams, S., Chatterjee, D., & Gerlai, R. (2015). Chronic social isolation affects thigmotaxis and whole-brain serotonin levels in adult zebrafish. *Behavioural Brain Research*, 292, 283–287.
- Shao, Y., Yan, G., Xuan, Y., Peng, H., Huang, Q. J., Wu, R., & Xu, H. (2015). Chronic social isolation decreases glutamate and glutamine levels and induces oxidative stress in the rat hippocampus. *Behavioural Brain Research*, 282, 201–208.
- Shpigler, H. Y., Saul, M. C., Corona, F., Block, L., Ahmed, A. C., Zhao, S. D., & Robinson, G. E. (2017). Deep evolutionary conservation of autism-related genes. *Proceedings of the National Academy of Sciences USA*, 114, 9653–9658.
- Silva-Gomez, A. B., Rojas, D., Juarez, I., & Flores, G. (2003). Decreased dendritic spine density on prefrontal cortical and hippocampal pyramidal neurons in postweaning social isolation rats. *Brain Research*, 983, 128–136.
- 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.
- Stevenson, P. A., & Rillich, J. (2013). Isolation associated aggression—a consequence of recovery from defeat in a territorial animal. *PLoS One*, 8, e74965.

- Szczuka, A., Korczyńska, J., Kieruzel, M., & Godzińska, E. J. (2011). The effects of presence of homo- and allospecific nestmates on survivorship of isolated workers of the obligatory slave-making amazon ant species (*Polyergus rufescens*) and their slaves (*Formica fusca*) (Hymenoptera: Formicidae). *Sociobiology*, *57*, 361–376.
- Szczuka, A., Korczyńska, J., Wnuk, A., Symonowicz, B., Gonzalez Szwacka, A., Mazurkiewicz, P., Kostowski, W., & Godzińska, E. J. (2013). The effects of serotonin, dopamine, octopamine and tyramine on behavior of workers of the ant *Formica polyctena* during dyadic aggression tests. *Acta Neurobiologiae Experimentalis*, *73*, 495–520.
- Theis, F. J., Ugelvig, L. V., Marr, C., & Cremer, S. (2015). Opposing effects of allogrooming on disease transmission in ant societies. *Philosophical Transactions of the Royal Society B*, *370*, 20140108.
- Tian, L., Preisser, E. L., Haynes, K. F., & Zhou, X. (2017). Social buffering in a eusocial invertebrate: termite soldiers reduce the lethal impact of competitor cues on workers. *Ecology*, *98*, 952–960.
- Toxopeus, J., Jakobs, R., Ferguson, L. V., Garipey, T. D., & Sinclair, B. J. (2016). Reproductive arrest and stress resistance in winter-acclimated *Drosophila suzukii*. *Journal of Insect Physiology*, *89*, 37–51.
- Tragust, S., Mitteregger, B., Barone, V., Konrad, M., Ugelvig, L. V., & Cremer, S. (2013). Ants disinfect fungus-exposed brood by oral uptake and spread of their poison. *Current Biology*, *23*, 76–82.
- Vavricka, C. J., Han, Q., Mehere, P., Ding, H., Christensen, B. M., & Li, J. (2014). Tyrosine metabolic enzymes from insects and mammals: a comparative perspective. *Insect Science*, *21*, 13–19.
- Vleugels, R., Verlinden, H., & Vanden Broeck, J. (2015). Serotonin, serotonin receptors and their actions in insects. *Neurotransmitter*, *2*, 1–14.
- Wagner-Ziemka, A., Gonzalez Szwacka, A., Korczyńska, J., Kieruzel, M., Fialkowska, B., & Godzińska, E. J. (2008). Comparison of the behavior of nurses and foragers of the carpenter ant, *Camponotus melanocnemis*, during dyadic nestmate reunion tests carried out after a period of social isolation (Hymenoptera: Formicidae). *Sociobiology*, *52*, 667–702.
- Wang, L., Xu, Y., Di, Z., & Roehner, B. M. (2016). Effect of isolation on life expectancy of red imported fire ant *Solenopsis invicta* and tephritid fruit fly *Bactrocera dorsalis*. *Acta Ecologica Sinica*, *36*, 252–255.
- Weiss, I. C., Pryce, C. R., Jongen-Rêlo, A. L., Nanz-Bahr, N. I., & Feldon, J. (2004). Effect of social isolation on stress-related behavioural and neuroendocrine state in the rat. *Behavioural Brain Research*, *152*, 279–295.
- Westhus, C., Ugelvig, L. V., Tourdot, E., Heinze, J., Doums, C., & Cremer, S. (2014). Increased grooming after repeated brood care provides sanitary benefits in a clonal ant. *Behavioral Ecology & Sociobiology*, *68*, 1701–1710.
- Wise, D. A. (1974). Aggression in the female golden hamster: Effects of reproductive state and social isolation. *Hormones & Behavior*, *5*, 235–250.
- Wu, W., Yamaura, T., Murakami, K., Murata, J., Matsumoto, K., Watanabe, H., & Saiki, I. (2000). Social isolation stress enhanced liver metastasis of murine colon 26–L5 carcinoma cells by suppressing immune responses in mice. *Life Sciences*, *66*, 1827–1838.
- Yang, N., Xie, W., Jones, C. M., Bass, C., Jiao, X., Yang, X. et al (2013). Transcriptome profiling of the whitefly *Bemisia tabaci* reveals stage-specific gene expression signatures for thiamethoxam resistance. *Insect Molecular Biology*, *22*, 485–496.
- Yocum, G. D., Childers, A. K., Rinehart, J. P., Rajamohan, A., Pitts-Singer, T. L., Greenlee, K. J., & Bowsher, J. H. (2018). Environmental history impacts gene expression during diapause development in the alfalfa leafcutting bee, *Megachile rotundata*. *Journal of Experimental Biology*, *221*, jeb173443.
- Yuan, K., Yuan, F. H., He, H. H., Bi, H. T., Weng, S. P., He, J. G., & Chen, Y. H. (2017). Heat shock 70 kDa protein cognate 5 involved in WSSV toleration of *Litopenaeus vannamei*. *Developmental & Comparative Immunology*, *72*, 9–20.
- Zavialov, A. V., Gracia, E., Glaichenhaus, N., Franco, R., Zavialov, A. V., & Lauvau, G. (2010). Human adenosine deaminase 2 induces differentiation of monocytes into macrophages and stimulates proliferation of T helper cells and macrophages. *Journal of Leukocyte Biology*, *88*, 279–290.
- Zhang, B. Z., Kong, F. C., Wang, H. T., Gao, X. W., Zeng, X. N., & Shi, X. Y. (2016). Insecticide induction of O-demethylase activity and expression of cytochrome P450 genes in the red imported fire ant (*Solenopsis invicta* Buren). *Journal of Integrative Agriculture*, *15*, 135–144.
- Zhao, X., Sun, L., Jia, H., Meng, Q., Wu, S., Li, N., & He, S. (2009). Isolation rearing induces social and emotional function abnormalities and alters glutamate and neurodevelopment-related gene expression in rats. *Progress in Neuropsychopharmacology & Biological Psychiatry*, *33*, 1173–1177.
- Zlatković, J., Bernardi, R. E., & Filipović, D. (2014). Protective effect of Hsp70i against chronic social isolation stress in the rat hippocampus. *Journal of Neural Transmission*, *121*, 3–14.
- Zlatković, J., Todorović, N., Bošković, M., Pajović, S. B., Demajo, M., & Filipović, D. (2014). Different susceptibility of prefrontal cortex and hippocampus to oxidative stress following chronic social isolation stress. *Molecular & Cellular Biochemistry*, *393*, 43–57.

## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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