Social isolation causes mortality by disrupting energy homeostasis in ants

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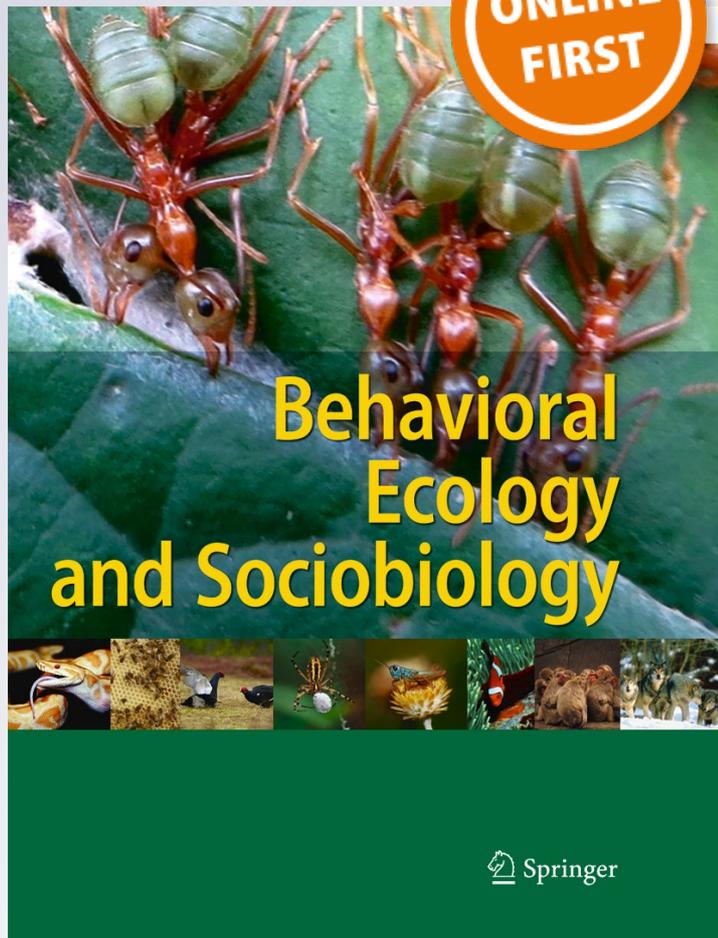# Social isolation causes mortality by disrupting energy homeostasis in ants

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Abstract Social deprivation can have negative effects on the lives of social animals, including humans, yet little is known about the mechanisms by which social withdrawal affects animal health. Here we show that in the carpenter ant *Camponotus fellah*, socially isolated workers have a greatly reduced life span relative to ants kept in groups of ten individuals. By using a new tracking system, we found that social isolation resulted in important behavioral changes and greatly increased locomotor activity. The higher activity of single ants and their increased propensity to leave the nest to move along the walls suggested that the increased mortality of isolated ants might stem from an imbalance of energy income and expenditure. This view was supported by the finding that while isolated ants ingested the same amount of food as grouped ants, they retained food in the crop, hence preventing its use as an energy source. Moreover, the difference in life span between single and grouped individuals vanished when ants were not fed. This study thus underlines the role of social interactions as key regulators of energy balance, which ultimately affects aging and health in a highly social organism.

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Introduction

Social interactions have a great impact on the health of animals. Social deprivation and isolation can lead to serious health problems through behavioral, psychosocial, and physiological pathways (Cacioppo and Hawkley 2009; Umberson and Montez 2010). For example, in social mammals, separation from conspecifics promotes the progression of diseases, such as obesity, type 2 diabetes (Nonogaki et al. 2007), cardiovascular disorders (Rozanski et al. 1999), or cerebrovascular disorders (Karelina et al. 2009; Venna et al. 2014) and induces high levels of stress response (Matsumoto et al. 2005; Wiberg and Grice 1963). Moreover, social isolation is one of the risk factors associated with reduced life span in various animals including humans (Boulay et al. 1999; Holt-Lunstad et al. 2010; House et al. 1988; Ruan and Wu 2008; Yang et al. 2013). These observations suggest that social interactions influence not only the behavior but also the physiology of social organisms, but there is little information on the mechanisms by which this occurs. Such information is important in order to gain a better understanding of the interplay between social interactions and health.

Social insects, in particular ants, are ideal models for studying how isolation affects an individual's well-being because interactions between individuals are a key component of their

social organization. Ants always live in groups, and survival of the workers is directly linked to survival of the colony. Workers are unable to establish a colony on their own and require a queen to ensure reproduction. Conversely, queens require workers to collect food and maintain the colony. Colonies can be composed of tens to millions of individuals and show a marked division of labor between workers (Mersch et al. 2013; Smith et al. 2008; Wilson and Hölldobler 1990). Each worker engages in different tasks, such as foraging, nest construction, and nursing. Foragers ingest food outside of the nest, store it in their crop (a thin-walled social sac where ants store food to share it with nestmates), and transfer it to their nestmates via trophallaxis, a mouth-to-mouth exchange of fluids among nestmates which allows equilibration of colony's energy state (Wilson and Hölldobler 1990). Workers are able to flexibly switch their repertoire of labor depending on colony demands (Huang and Robinson 1992; Robinson et al. 1989; Seid and Traniello 2006). Thus, the behavioral pattern of each worker, their nutrient status, and their physiology are dependent on complex regulatory mechanisms acting at the level of the colony. This social responsiveness is possible because workers have complex social interactions which, in aggregate, provide precise information on their social environment, including the current division of labor and colony size.

Several studies have shown that health and aging of social insects are affected by their social interactions (Boulay et al. 1999; Roger and Pain 1966; Sitbon 1967). Isolation can lead to high mortality rates within a few days. The reasons for the mortality of isolated individuals remain unknown. Interestingly, the mortality rate of workers is decreased when they are kept with a few other workers, and there is a positive group effect on worker survival between 2 and 20 individuals (Arnold 1976; Grassé and Chauvin 1944). In this study, we take advantage of this group effect to quantify how the social environment influences an individual's behavior and to investigate which mechanisms cause the mortality of socially deprived ants. We used minor workers from seven laboratory-reared colonies of the carpenter ant *Camponotus fellah*. Workers were either kept in isolation with or without brood, in groups of two, or in groups of ten. We found that isolated ants have a strikingly reduced life span compared to ants kept with nestmates, confirming the findings of a previous study (Boulay et al. 1999). Monitoring ant behavior with a tracking system (Mersch et al. 2013) revealed that the isolated ants exhibited elevated activity just after the onset of social isolation, continuously walking without any rest. Furthermore, while isolated ants ingested similar amounts of food as the ants kept in groups, they retained food in the crop instead of digesting it. Thus, not only behavior but also the physiological functions of the digestive system were affected by social deprivation.

Material and methods

Ants

Colonies were initiated from queens collected after a mating flight in March 2007 in Tel Aviv, Israel. The ants were reared in the laboratory under controlled conditions (12:12 LD, 30 °C, 60 % RH). For all experiments, we used workers from seven queenright colonies that had approximately 300 workers and one queen. We used only minor workers in which body size was about 8 mm.

Social environment setting

Workers were separated from the mother colonies and reared in plastic boxes (105×87 mm), which contained food and water ad libitum and a light-shielded nest box (Fig. 1a). We had four treatments, single worker (single, $N=60$), groups of two workers (dyad, $N=82$), groups of ten workers (grouped, $N=131$), and single workers with 3–4 medium-sized larvae (brood, $N=28$) originating from the same mother colony. Workers of all treatments were kept at 30 °C, 60 % humidity under 12 h light-12 h dark cycles. In the food deprivation experiment, starved ants also had access to water.

Survival analysis

We analyzed the survival of ants in different social environments with proportional hazards regression models (PROC PHREG) in SAS (SAS Inc.). In order to account for the covariance between individuals from the same source colony or from the same box, models included "frailty" (random) effects for each colony and box id. Survival curves are plotted after collapsing the replicates within each colony and social condition combination into one survival curve. Standard error therefore reflects colony-to-colony variance for these curves within each treatment. Similarly, in Figs. 1c and 4b, we collapsed every unique box to one median survival value. We calculated an average of these median survival times for each colony, which we then analyzed with a general linear mixed model procedure (GLMM) using R (R 2.14.1) with one fixed factor (social treatment) and one random factor (colony of origin).

Behavior tracking system

We used the tracking system developed by Mersch et al. (Mersch et al. 2013) to compare the behavior of single ants and ants kept in groups of ten individuals. Ants were tagged with unique matrix codes (1.6 mm side length) while immobilized in a cold room (4 °C). Tracking experiments were performed under controlled conditions (12:12 LD, 30 °C, 60 % RH). From the video, we manually defined the
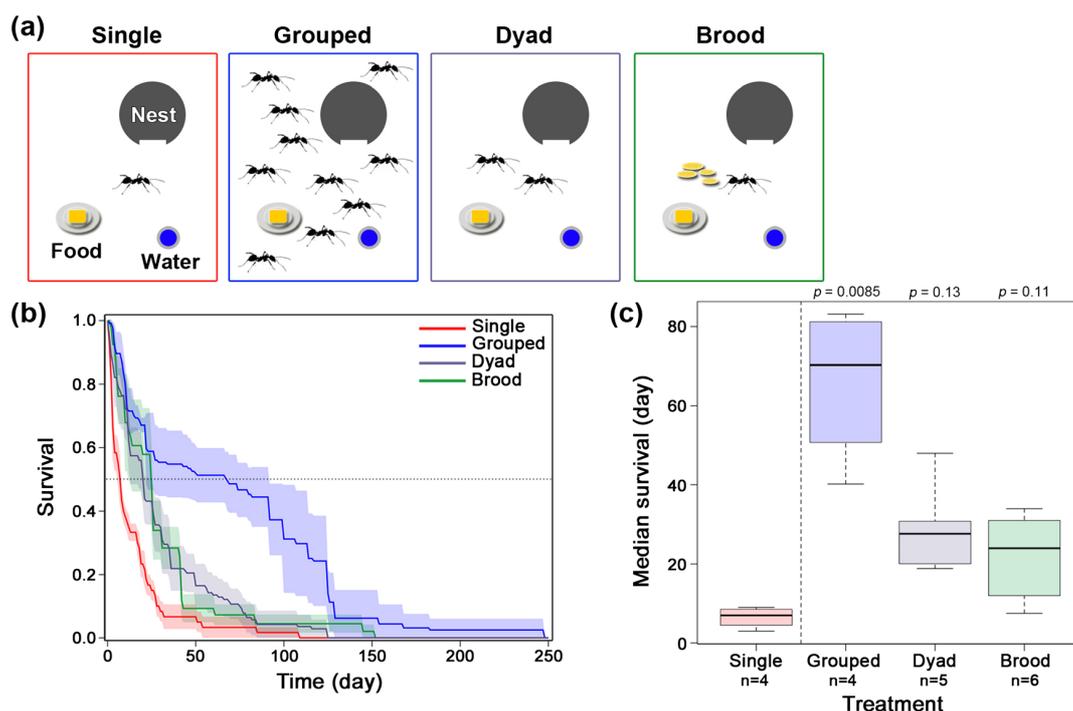
Fig. 1 Social environment affects the fitness of ants. **a** Schematic diagram. Workers were alone (single), in groups of two individuals (dyad), in groups of ten individuals (grouped), or alone with three or four larval brood (brood) in the test box which contained food, water, and the dark chamber. **b** Survival curves (*solid line*)±S.E. (*shaded region*) for single (*red*), dyad (*purple*), grouped (*blue*), and brood (*green*) conditions. Single ants showed significantly reduced life span relative to all other treatments, *p* <0.0001. **c** The median survival time for each treatment. Sixty (single), 14 (grouped), 41 (dyad), and 28 (brood) independent boxes were measured, and a median survival obtained for each box. These values were collapsed by averaging the medians for the different boxes of an individual colony, to give one median survival per colony. *Box plot* shows medians (*center lines*) and interquartile ranges (*boxes*); *whiskers* indicate the minimum and maximum values. *P* values represent comparisons against single treatment and were tested using GLMM with Tukey's post hoc test (no data transformation)

time of death for each ant as the time when the ant fell over and then lay on the ground, immobile.

Tracking data processing

The tracking data obtained were post-processed as described previously (Mersch et al. 2013). To automatically infer the time spent by each ant near the wall and near the food, we specified the coordinates of the two regions of interest on the images. For each ant, we then calculated the total duration spent in each region of interest in every 24 h interval. Because we could not film the ants inside the nest, we estimated the time spent inside the nest as the sum of the differences between each time of entry and time of exit of the nest. We inferred entry and exit time from the position and orientation of each ant. An ant entered the nest when the distance between the ant and the nest entrance was less than 300 pixels, and the ant was facing the entrance (i.e., the ant orientation was perpendicular to the entrance: $90 \pm 10°$) for at least 5 s, and then remained undetected for at least 5 s. Exit times were assigned to first detections after the ant had entered the nest. The distance an ant traveled was estimated as the sum of Euclidean

distances between all subsequent positions. If the ant was undetected for part of its trajectory, we took the shortest line that connected the two points of detection.

Quantification of the amount of food ingested and digested

To estimate the amount of food an ant had ingested and was digesting, we randomly picked up workers and fed them with dyed sucrose water and quantified the amount of dye as a proxy for the amount of food in the crop and digestive tract of the ant. We dyed 100 mM sucrose water with 5 % erioglaucine (FD & C Blue No. 1, Sigma). Ants were fed with dyed food for 6 h and then with 100 mM sucrose water for the following 24 h. We quantified the amount of blue dye at three time points: immediately after dye-feeding, 24 h after dye-feeding, and 48 h after dye-feeding. The abdomen of each ant was dissected, and either crop and the digestive tract (immediately and 24 h after feeding) or only the digestive tract (24 and 48 h after feeding) was kept for dye quantification. The digestive tract, or the digestive tract with crop, was homogenized in 400 µl of 0.1 mol/L of phosphate buffer (pH

7.2). The amount of blue dye was measured at 633 nm in a spectrophotometer.

Statistical analysis

The behavior parameters and the amount of food were compared among treatments with GLMM using R (R 2.14.1). For the analysis of distance traveled (Fig. 2b) and time spent in the wall region (Fig. 2c), we calculated the mean value in each grouped condition and fit a model with one fixed factor (treatment) and one random factor (colony of origin). For the analysis of time spent in the nest region (Fig. 2d) and food consumption (Fig. 3b, c), we used the data from each ant and fit a GLMM with treatment as a fixed factor and both box and colony of origin as random factors to account for any variation between experimental boxes and colonies. The normality of residuals was checked with Shapiro-Wilk tests. When the residuals were not normally distributed, we applied square root transformation to the data, which normalized the residuals.

Results

Reduced life span in social isolation

To investigate the effect of social environment on life span, we maintained workers under four different treatments: three treatments consisted of workers kept either in isolation (single), in groups of two (dyad), or groups of ten (grouped). In the fourth treatment, single workers were kept together with 3–4 larvae (brood). All experiments were performed in plastic boxes containing food, water, and a freely accessible dark nest chamber (Fig. 1a). We selected minor workers, the smallest members of the worker caste (body size ca. 8 mm), and only used individuals with black cuticle coloration to ensure that they were at least 2 months old.

There was a strong effect of social environment on life span (Wald χ^2=30.9, p<0.0001). In the experiments without larvae, isolated single ants had the shortest life span (mean±se= 6.5±1.3 days) while ants in groups (66±9.8 days) had the longest life span (single versus grouped; Wald χ^2=30.8, p<0.0001; Fig. 1b, c). Ants in the dyad treatment had an
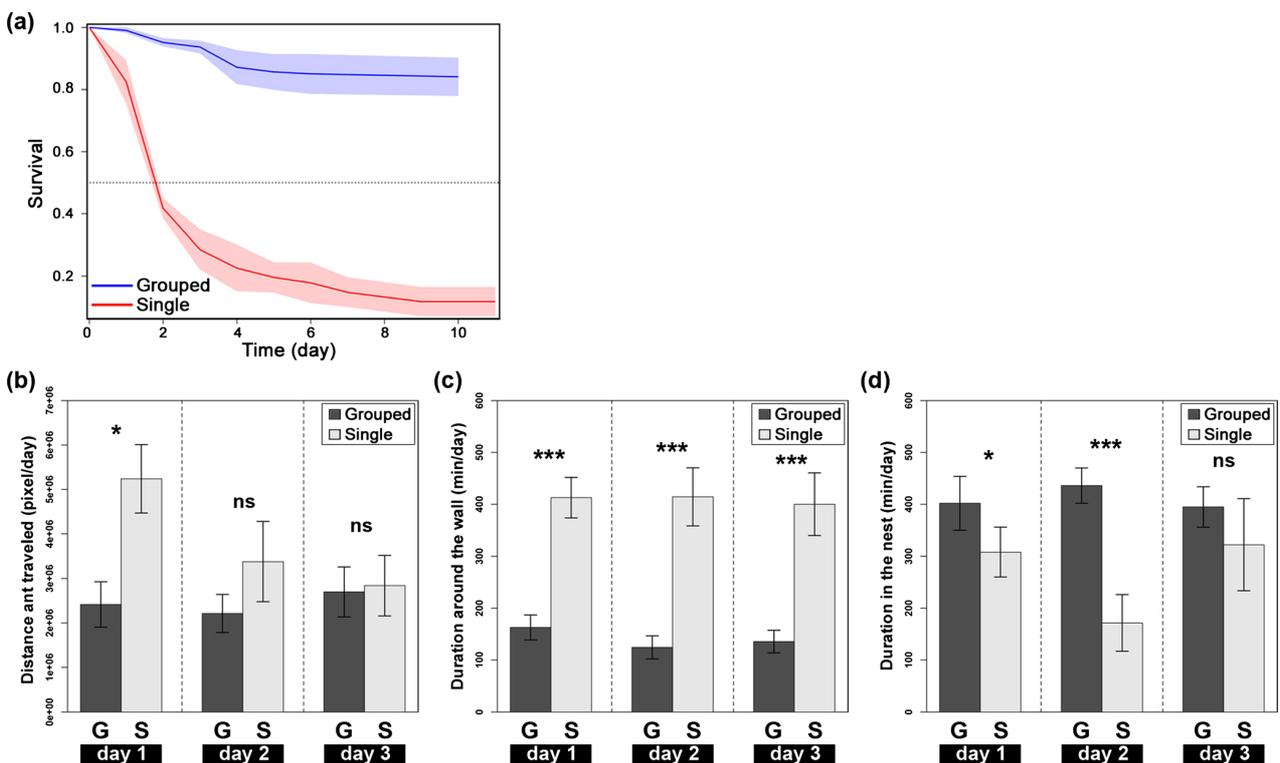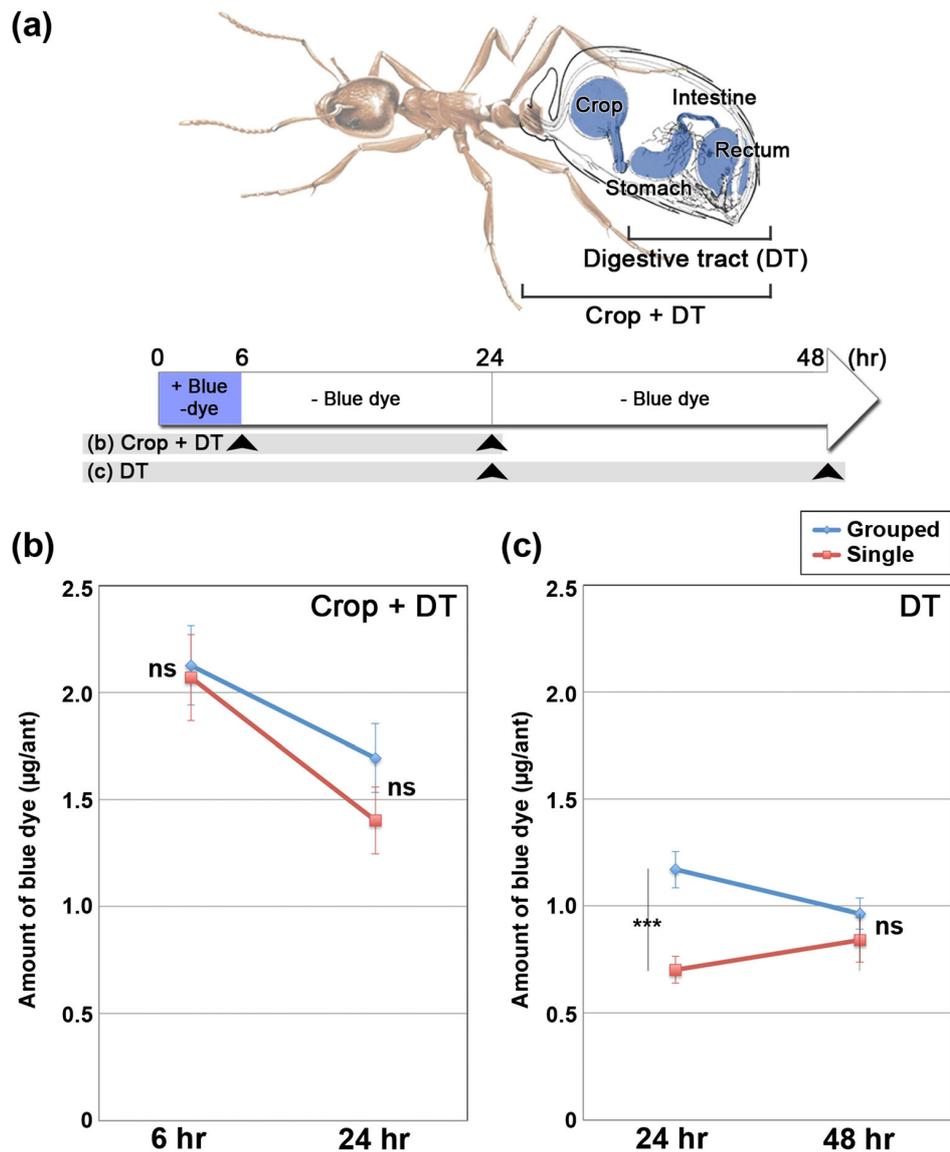

Fig. 2 Hyperactive behavior in social isolation. **a** Survival curves (*solid line*)±S.E. (*shaded region*) for single (*red*) and grouped (*blue*) conditions in behavioral tracking system. **b–d** Single ants showed hyperactive behavior relative to grouped ants. The **b** distance traveled, the **c** duration in the wall region, and the **d** duration inside the nest (mean± se) were calculated in single (*light gray*; n=44, 22, and 15 from days 1 to 3) and grouped (*dark gray*, n=15) conditions for the first 3 days of the tracking experiment. The effect of treatment on behavior was tested using GLMM (**b–d**). The **b** distance ant traveled and the duration in the nest were square-root transformed. No data transformation in **c**. ns; p>0.05; *p<0.05; **p<0.01; ***p<0.001

Fig. 3 Impairment of digestive system in social isolation. **a** Experimental procedure for the quantification of food intake. Ants were kept with sucrose water with blue dye for 6 h and with sucrose water for the following 2 days. The abdomen which contains the crop and digestive tract (*DT*) or only the DT were dissected to quantify the amount of food ants ingested at **b** 6 or 24 h and digested at **c** 24 and 48 h. **b** The amount of blue dye ingested (mean±se) in grouped (*blue*) and single ant (*red*) immediately or 24 h after feeding is shown. The sample sizes are 29 single and 28 grouped individuals at 6 h and 32 single and 39 grouped individuals at 24 h. **c** The amount of blue dye in digestive tract 24 or 48 h after feeding. The sample sizes are 38 single and 36 grouped individuals at 24 h and 31 single and 30 individuals at 48 h. The effect of treatment on the amount of food was tested using GLMM (no data transformation). ns; $p>0.05$; ***$p<0.001$

intermediate life span (29±5.2 days), significantly different from both the single ant (Wald $\chi^2=26.6$, $p<0.0001$) and grouped treatments (Wald $\chi^2=8$, $p=0.005$). The presence of larvae also strongly influenced life span. The median survival of single ants kept with larvae (22±4.3 days) was more than three times higher than that of single ants kept without larvae (Wald $\chi^2=25.4$, $p<0.0001$), demonstrating the effect of brood presence on ant longevity.

To investigate whether the reduced life span of socially deprived ants depended on age, we color-coded ants over the course of 7 months to obtain six age classes (2 to 7-month-old individuals) and repeated the experiment under the conditions that most affected life span (i.e., single ants versus groups of ten individuals). As before, socially deprived ants had shorter life spans than ants in groups (Wald $\chi^2=11.7$,

$p=0.0006$). Age of ants also influenced their longevity with younger individuals surviving longer (Wald $\chi^2=11.3$, $p=0.0008$). There was, however, no significant interaction between social treatment and age (Wald $\chi^2=2.35$, $p=0.13$, Fig. S1), indicating that social isolation has similar negative effects on life span regardless of worker age.

Hyperactive behavior in social isolation

To determine whether decreased life span under isolated conditions was associated with a behavioral stress response, we monitored the behavior of ants with an automated video tracking system (Mersch et al. 2013) over the course of 10 days. Every ant carried an individual tag, allowing one to precisely infer the behavior of each individual in an unbiased manner.

Consistent with the results of our previous experiments, we found that single ants had a much shorter life span than ants in groups (Wald χ^2=25.8, $p<0.0001$, Fig. 2a). The average life span of workers was lower in this experiment than in the first experiment (Wald χ^2=16.4, $p<0.0001$), probably because of the additional stress incurred in the tracking setup (e.g., application of the tag on the thorax). Because of the short median survival times of single ants, we analyzed their behavior only during the first 3 days at which time 40.8 % of single ants were still alive.

Social isolation resulted in significantly increased locomotor activity (Fig. 2b). On the first day after isolation, the total distance covered by single ants was more than twice that of ants kept in groups (χ^2=4.2, p=0.041, Fig. 2b). Visual inspection of the videos further revealed clear behavioral differences, including single ants spending considerably more time walking near the walls of the box than ants in groups. A quantitative analysis of the spatial location during the first 3 days of video tracking showed that single ants spent almost three times as much time near the wall (Fig. 2c) and less time in the nest (Fig. 2d) than grouped ants. Together, the higher locomotor activity of single ants and their increased propensity to leave the nest to move along the walls led us to hypothesize that the increased mortality of isolated ants may stem from an imbalance of energy income and expenditure.

Impairment of digestion process in isolation

To investigate whether social isolation negatively affected the process of food digestion, we fed isolated workers and ants kept in groups of ten during 6 h with blue-colored sugar water and then dissected them to extract the crop and the digestive tract (DT; ventriculus, intestine, and rectum) (Fig. 3a). To determine whether isolated and grouped ants differed in the amount of food ingested, and how the food is processed, we dissected workers either immediately after feeding, 24 h after feeding, or 48 h after feeding. For each ant, we quantified the amount of blue color using spectrophotometry. The amount of food ingested (crop+digestive tract) was not significantly different between isolated ants and ants in groups immediately after feeding ($N_{isolated}$=29 ants, $N_{grouped}$=28 ants, χ^2=0.037, p=0.85, Fig. 3b) or 24 h after feeding ($N_{isolated}$=32 ants, $N_{grouped}$=39 ants, χ^2=0.36, p=0.55, Fig. 3b). However, social isolation negatively impacted the process of food digestion. The amount of food in the digestive tract was significantly lower in isolated ants than ants in groups 24 h after feeding ($N_{isolated}$=38 ants, $N_{grouped}$=36 ants, χ^2=19.19, $p<0.0001$, Fig. 3c). This difference disappeared after 48 h ($N_{isolated}$=31 ants, $N_{grouped}$=30 ants, χ^2=1.48, p=0.22, Fig. 3c). Single ants thus digested food at a slower rate than grouped ants which was also reflected by a significant interaction (F=4.37, df=89, p=0.039) between treatment and time (24 versus 48 h) in the amount of food present in the digestive tract (Fig. 3c). In ants,

the transfer of food from the crop to the ventriculus is normally prevented by a portal valve (Eisner and Happ 1962), and food transfer requires active proventriculus pumping (Hansen and Klotz 2005). Consequently, social isolation appears to interfere with the pumping process, reducing the amount of food transferred to the digestive tract and therefore the amount of energy available to isolated ants.

To understand whether this reduced energy supply combined with higher locomotor activity could be a determinant of the elevated mortality of isolated ants, we tested the effect of food deprivation on the life span of isolated and grouped ants. If energy imbalance was the main factor responsible for the shorter life span of isolated workers, one would predict that food deprivation has a significant effect on life span and that this effect is more important for grouped ants than for single ants. In line with these predictions, we found that food deprivation significantly shortened the life span of both isolated and grouped ants (fed versus starved, Wald χ^2=36.8, $p<0.0001$, Fig. 4a, b). However, the decrease in life span was particularly marked for grouped ants (food status×social environment interaction, Wald χ^2=7.1, p=0.008), and there was no difference in life span between single and grouped ants under starvation (Wald χ^2=0.54, p=0.46). Overall, these data support the view that variation in life span between single and grouped ants is associated with energy imbalance.

Discussion

Many animals fare poorly when they are isolated from their social group. The effect of social deprivation has historically been studied in social insects because of their striking phenotype, a shortened life span in social isolation (Arnold 1976; Boulay et al. 1999; Grassé and Chauvin 1944). These experiments were performed in artificial conditions where workers were forcibly removed from the colony. However, shortened life span was also observed when moribund ants left the colony on their own (Heinze and Walter 2010), suggesting that shortened life span is a robust phenotype of social insects under social isolation. However, the mechanistic explanation for how social withdrawal reduces ant life span is unknown. The automated video tracking setup we employed allowed us to quantify individual behavior of both isolated individuals and individuals in groups. We found that social isolation was associated with behavioral abnormalities. Notably, isolated ants exhibited higher locomotor activity and a much greater propensity to leave the nest and walk near the walls of the box than individuals in groups.

Because of their hyperactive behavioral pattern, single ants faced an acute increased energy demand. Therefore, we hypothesized that the imbalance of energy supply and consumption causes the mortality of isolated ants. So far, only a few
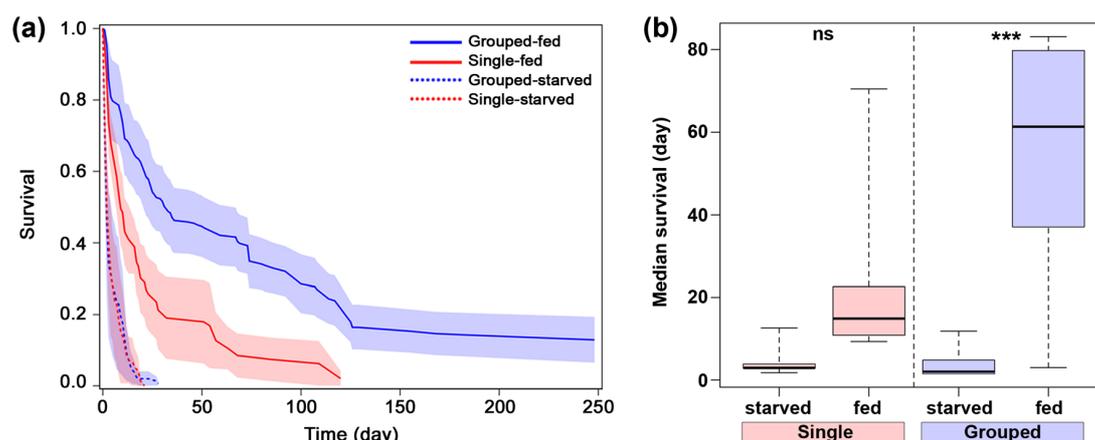
Fig. 4 Food deprivation reduces the life span in both grouped and isolated conditions. **a** Survival curves (*solid line*)±S.E. (*shaded region*) for single-fed (*red line*), grouped-fed (*blue line*), single-starved (*red dotted line*), and grouped-starved (*blue dotted line*) conditions. **b** The median survival time for each treatment. Ninety-eight (single-starved), 76 (single-fed), 14 (grouped-starved), and 19 (grouped-fed) independent boxes were measured and a median survival obtained for each box. These values were collapsed by averaging the medians for the different boxes of an individual colony, to give one median survival per colony. *Box plot* shows medians (*center lines*) and interquartile ranges (*boxes*); *whiskers* indicate the minimum and maximum values. The effect of feeding condition on longevity was tested using GLMM (no data transformation). ns; $p > 0.05$; ***$p < 0.001$

studies have investigated whether social environment affects food intake in social insects. Sitbon showed that the total amount of sugar in worker honeybees was not significantly different between individuals that were kept isolated or in groups of 30 (Sitbon 1967; Sitbon 1968a; Sitbon 1968b). In flies, though social experience can have positive effects on longevity for some mutants (Ruan and Wu 2008), the size of the group does not influence the feeding rate of individuals (Ja et al. 2007). In this study, we also found that the amount of food ingested did not differ between isolated and grouped ants. However, our analysis of the digestive process itself revealed that digestion is less efficient in isolated ants. There is currently no similar data in other social animals. In highly social animals, it is known that subsets of individuals with social or behavioral deficits such as autism spectrum disorders display gastrointestinal abnormalities (Buie et al. 2010; Coury et al. 2012; Hsiao et al. 2013). Our finding that the social environment directly affects the process of digestion in social insects is interesting in that respect, as it suggests a deep evolutionary link between social behavior and the modulation of gut-brain communication.

Our findings provide evidence that food intake alone does not determine energy supply of individual ants. *C. fellah* requires social interaction with nestmates to promote food flow from the crop to the digestive tract. Further study will be necessary to fully understand the mechanisms by which the social environment regulates digestion. One hypothesis is that the digestive process is affected by trophallaxis, which is an oral sharing of regurgitated food and fundamental to social organization in ants. After foragers store food in the crop and come back to the nest, the majority of workers quickly receive the food within an hour (Buffin et al. 2009; Howard

and Tschinkel 1981; Markin 1970). Most workers always acquire food via mouth-to-mouth feeding, except for foragers who ingest food outside the nest by themselves. Therefore, trophallaxis fluid may function to modify the food contents or provide some additional compound that stimulates digestion. Alternatively, the physical interaction between nestmates might activate neural pathways, which promote gastrointestinal activity. Interestingly, the presence of larvae elongated the life span of isolated ants. This effect of larvae could also have been mediated by direct trophic effects (e.g., by regurgitating food to the workers) or via physical interactions. A positive effect of larvae presence on worker life span has also been reported in the ant *Temnothorax nylanderi* (Modlmeier et al. 2013). Understanding more precisely the relationship between gastrointestinal function and social environment will require the use of methods to measure food flow in a noninvasive way (e.g., by scintigraphy (Buffin et al. 2009) or defecation rate (Cognigni et al. 2011)).

Our food deprivation experiments also support the view that reduced energy supply combined with higher locomotor activity is critical determinant of the elevated mortality of isolated ants. When ants were deprived of food, there was no difference in the life span of single and grouped ants. These results differ from those obtained in *Leptothorax nylanderi* where, under starvation, single ants showed reduced life span relative to ants kept in groups of three (Modlmeier et al. 2013). A possible explanation for this difference is that *Temnothorax* ants are more resistant to starvation because they live in temperate zones. Consistent with this view, our experiments showed that the life span of *C. fellah* was very low under starvation. Because Modlmeier et al. (2013) did not study life span of fed workers in *L. nylanderi*, it is unfortunately not

possible to compare the interaction between starvation and group size between the two studies.

Our results raise the important question of how social bonding regulates the behavior and physiology of ants. Early work by Grassé and Chauvin revealed important variation across species in the effect of social isolation with significant effects in the honeybee, termites, and ants, but not in paper wasps (Grassé and Chauvin 1944). We also found significant differences between two *Camponotus* species. When workers of *Camponotus floridanus* were kept in groups of ten individuals, their life span was similarly reduced as for ants kept in isolation (A. Koto and L. Keller, unpublished). Interestingly, *C. floridanus* forms larger colonies than *C. fellah*, raising the possibility that species with larger colonies suffer more readily from reduced group size than species with smaller colony sizes. Moreover, the effect of social isolation may also differ according to the life cycle of a colony. In many ant species, colonies are initiated by independent colony founding whereby a queen initiates a colony alone after colony founding. In *C. fellah*, it takes about 8 weeks before the first workers are produced, and, as in other ants, there is no evidence of high queen mortality during this period (Keller and Passera 1990; D. Mersch, unpublished). Thus, the negative effect of social isolation can vary greatly depending on the type of individual or part of colony life cycle considered.

Finally, an interesting issue relates to the physiological changes associated with social isolation. It has been reported that levels of octopamine are upregulated in isolated ants (Wada-Katsumata et al. 2011). Further investigation addressing differences in gene expression between single and grouped ants is likely to shed light on the molecular mechanisms by which social environment affects the health of individuals. We herein succeeded in quantifying the longevity, behavior, and physiological state of isolated ants, all of which are markedly different from the grouped social environment. These will be useful landmarks to identify genes mediating changes in behaviors and physiology in response to changes in social factors. The study of social isolation gives us insight into how social bonding regulates the behavior and physiology of ants in normal colony condition. From the quantitative analysis of social behavior and physiological state of each individual, our study reveals that energy imbalance is the main cause of high mortality in social isolation. Social interactions thus appear to be an essential regulator of energy homeostasis that is responsible for both individual- and colony-level health.

References

Arnold G (1976) Mechanisms in the group effect of honeybees. C R Acad Sci Hebd Seances Acad Sci D 283:1433–1435

Boulay R, Quagebeur M, Godzinska E, Lenoir A (1999) Social isolation in ants: evidence of its impact on survivorship and behavior in *Camponotus fellah* (Hymenoptera: Formicidae). Sociobiology 33: 111–124

Buffin A, Denis D, Van Simaeys G, Goldman S, Deneubourg JL (2009) Feeding and stocking up: radio-labelled food reveals exchange patterns in ants. PLoS One 4:e5919. doi:10.1371/journal.pone.0005919

Buie T et al (2010) Evaluation, diagnosis, and treatment of gastrointestinal disorders in individuals with ASDs: a consensus report. Pediatrics 125(Suppl 1):S1–S18. doi:10.1542/peds. 2009-1878C

Cacioppo JT, Hawkley LC (2009) Perceived social isolation and cognition. Trends Cogn Sci 13:447–454. doi:10.1016/j.tics.2009.06.005

Cognigni P, Bailey AP, Miguel-Aliaga I (2011) Enteric neurons and systemic signals couple nutritional and reproductive status with intestinal homeostasis. Cell Metab 13:92–104. doi:10.1016/j.cmet.2010.12.010

Coury DL et al (2012) Gastrointestinal conditions in children with autism spectrum disorder: developing a research agenda. Pediatrics 130(Suppl 2):S160–S168. doi:10.1542/peds. 2012-0900N

Eisner T, Happ G (1962) The infrabuccal pocket of a formicine ant: a social filtration device. Psyche 69:107–116

Grassé P, Chauvin R (1944) L'effet de group et de la survie des neutres dans les sociétées d'insectes. Rev Sci 82:261–264

Hansen L, Klotz J (2005) Carpenter Ants of the United States and Canada. Comstock Pub Assoc

Heinze J, Walter B (2010) Moribund ants leave their nests to die in social isolation. Curr Biol 20:249–252. doi:10.1016/j.cub.2009.12.031

Holt-Lunstad J, Smith TB, Layton JB (2010) Social relationships and mortality risk: a meta-analytic review. PLoS Med 7:e1000316. doi:10.1371/journal.pmed.1000316

House JS, Landis KR, Umberson D (1988) Social relationships and health. Science 241:540–545

Howard D, Tschinkel W (1981) The flow of food in colonies of the fire ant, *Solenopsis invicta*: a multifactorial study. Physiol Entomol 6:297–306

Hsiao EY et al (2013) Microbiota modulate behavioral and physiological abnormalities associated with neurodevelopmental disorders. Cell 155:1451–1463. doi:10.1016/j.cell.2013.11.024

Huang ZY, Robinson GE (1992) Honeybee colony integration: worker-worker interactions mediate hormonally regulated plasticity in division of labor. PNAS 89:11726–11729

Ja WW et al (2007) Prandiology of *Drosophila* and the CAFE assay. PNAS 104:8253–8256. doi:10.1073/pnas.0702726104

Karelina K, Norman GJ, Zhang N, Morris JS, Peng H, DeVries AC (2009) Social isolation alters neuroinflammatory response to stroke. PNAS 106:5895–5900. doi:10.1073/pnas.0810737106

Keller L, Passera L (1990) Fecundity of ant queens in relation to their age and the mode of colony founding. Insect Soc 37:116–130

Markin G (1970) Food distribution within laboratory colonies of the argentine ant, *Tridomyrmex humilis* (Mayr). Insect Soc 17:127–157

Matsumoto K, Pinna G, Puia G, Guidotti A, Costa E (2005) Social isolation stress-induced aggression in mice: a model to study the

pharmacology of neurosteroidogenesis. Stress 8:85–93. doi:10.1080/10253890500159022

Mersch DP, Crespi A, Keller L (2013) Tracking individuals shows spatial fidelity is a key regulator of ant social organization. Science 340:1090–1093. doi:10.1126/science.1234316

Modlmeier AP, Foitzik S, Scharf I (2013) Starvation endurance in the ant *Temnothorax nylanderi* depends on group size, body size and access to larvae. Physiol Entomol 38:89–94. doi:10.1111/Phen.12007

Nonogaki K, Nozue K, Oka Y (2007) Social isolation affects the development of obesity and type 2 diabetes in mice. Endocrinology 148:4658–4666. doi:10.1210/en.2007-0296

Robinson GE, Page RE Jr, Strambi C, Strambi A (1989) Hormonal and genetic control of behavioral integration in honey bee colonies. Science 246:109–112. doi:10.1126/science.246.4926.109

Roger G, Pain J (1966) L'influence de la reine d'abeille (*Apis mellifica* L.) sur le taux de mortalité des ouvrières accompagnatrices. Ann Abeille 9:5–36

Rozanski A, Blumenthal JA, Kaplan J (1999) Impact of psychological factors on the pathogenesis of cardiovascular disease and implications for therapy. Circulation 99:2192–2217

Ruan H, Wu CF (2008) Social interaction-mediated lifespan extension of *Drosophila* Cu/Zn superoxide dismutase mutants. PNAS 105:7506–7510. doi:10.1073/pnas.0711127105

Seid M, Traniello J (2006) Age-related repertoire expansion and division of labor in *Pheidole dentata* (Hymenoptera: Formicidae): a new perspective on temporal polyethism and behavioral plasticity in ants.

Behav Ecol Sociobiol 60:631–644. doi:10.1007/s00265-006-0207-z

Sitbon G (1967) L'effet de groupe et la mortalité des abeilles d'hiver et d'été, isolées et groupées. Ann Abeille 10:203–212

Sitbon G (1968a) Les sucres totaux chez l'abeille d'hiver en fonction de l'isolement. Insect Soc 15:37–44

Sitbon G (1968b) Teneur en eau et en azote chez l'abeille en fonction de l'isolement. Insect Soc 15:413–418

Smith CR, Toth AL, Suarez AV, Robinson GE (2008) Genetic and genomic analyses of the division of labour in insect societies. Nat Rev Genet 9:735–748. doi:10.1038/nrg2429

Umberson D, Montez JK (2010) Social relationships and health: a flashpoint for health policy. J Health Soc Behav 51(Suppl):S54–S66. doi:10.1177/0022146510383501

Venna VR, Xu Y, Doran SJ, Patrizz A, McCullough LD (2014) Social interaction plays a critical role in neurogenesis and recovery after stroke. Transt Psychiatry 4:e351. doi:10.1038/tp.2013.128

Wada-Katsumata A, Yamaoka R, Aonuma H (2011) Social interactions influence dopamine and octopamine homeostasis in the brain of the ant *Formica japonica*. J Exp Biol 214:1707–1713. doi:10.1242/jeb.051565

Wiberg GS, Grice HC (1963) Long-term isolation stress in rats. Science 142:507

Wilson E, Hölldobler B (1990) The ants. Springer, Berlin

Yang YC, McClintock MK, Kozloski M, Li T (2013) Social isolation and adult mortality: the role of chronic inflammation and sex differences. J Health Soc Behav 54:183–203. doi:10.1177/0022146513485244