

Moribund Ants Leave Their Nests to Die in Social Isolation

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Summary

Animal societies provide perfect conditions for the spread of infections and are therefore expected to employ mechanisms that reduce the probability of transmitting pathogens to group members [1–4]. Death in nature rarely results from old age but commonly results from diseases. Leaving one's group to die in seclusion might be an efficient way of minimizing the risk of infecting kin. Anecdotal observations of moribund individuals deserting from their groups exist for several species, including humans (e.g., [5]), but have rarely been substantiated by quantitative analysis. Furthermore, to confirm that dying in solitude has evolved because of its altruistic benefits requires refuting the alternative explanation of pathogen manipulation of host behavior. Here we show that workers of the ant *Temnothorax unifasciatus* dying from fungal infection, uninfected workers whose life expectancy was reduced by exposure to 95% CO₂ [6, 7], and workers dying spontaneously in observation colonies exhibited the same suite of behavior of isolating themselves from their nestmates days or hours before death. Actively leaving the nest and breaking off all social interactions thus occurred regardless of whether individuals were infected or not. Social withdrawal might be a commonly overlooked altruistic trait serving the inclusive fitness interests of dying individuals in social animals.

Results and Discussion

Because death in nature commonly results from infectious diseases or parasites, dying animals constitute a considerable risk for uninfected conspecifics. This is particularly the case in social animals, which usually live in more or less extended families and constantly engage in intimate interactions [8]. Both features greatly facilitate the spread of pathogens [3, 4]. Moribund individuals might reduce the probability of infecting family members by breaking off all social contact, leaving the group, and dying away from it in self-inflicted quarantine. Popular science and literature recount anecdotes about such behavior in several species of feral and domestic animals, including humans [5, 9–11]. However, such reports rarely stand up to scrutiny. Furthermore, it has been questioned whether death in solitude is indeed adaptive for the dying individual, which, through protecting family members from infection, increases its own inclusive fitness [12]. Instead, social withdrawal and death in isolation might constitute behavioral manipulations by the pathogen, which in this way promotes its own spread from group to group [11, 13, 14]. For example, social insects that are infected by fungi or are intermediate hosts of liver fluke larvae leave their nests and die on the tip

of grass stems or in other exposed sites. This obviously minimizes the rate of transmitting pathogens to nestmates, but it also increases the likelihood of fungal spores being dispersed by wind or liver flukes being taken up by a final host.

We investigated the behavior of workers of the ant *Temnothorax unifasciatus*, which died under controlled laboratory conditions. Our observations corroborate previous, scattered anecdotes about social withdrawal and death in isolation in group-living animals. In addition, the comparison among workers that died (1) from a fungal infection, (2) of unknown causes in unmanipulated colonies, or (3) because of exposure to CO₂ [6, 7] documents that this phenomenon is not necessarily a behavioral manipulation by a pathogen. Instead, it appears to be an active response of dying workers to the closeness of death. In all cases, moribund workers actively ceased social contact with their nestmates and left their nests hours or days before death without ever returning into it, regardless of whether they died from an infection or from other causes.

In total, 52 of 70 workers (74%) treated with spores of the entomopathogenic fungus *Metarhizium anisopliae* died within 10 days after the treatment (40%–100% per colony, median 80%). We excluded eight dying workers from the analysis because no spores grew on their corpses and they thus had died of unknown causes. Of the remaining 44 dying workers, at least 31 (70.5%) permanently left the nest 1–50 hr before their deaths (median 6 hr) and died outside in the foraging arena, away from adult nestmates and brood. Nine additional workers (20.5%) were found dead away from their nests in the early morning, but because we did not monitor their behavior overnight, we do not know whether they left the nest actively. Four workers (9%) died in the nest, and their corpses were removed by nestmates within 100 hr after their deaths. Spore-treated workers dying of unknown causes showed the same social withdrawal (four workers were observed leaving the nest, two workers were found dead outside the nest, and two died inside the nest).

A similar behavior of dying workers was observed in unmanipulated colonies. During behavioral investigations of 28 *T. unifasciatus* colonies with, in total, 1609 individually marked workers, we witnessed the spontaneous death of one worker each in 13 colonies. Twelve of these (92%) left the nest before they died. In contrast to fungus-infected workers, ants left the nests already between 1 and 15 days (median 3 days) before death, presumably because fungal infection results in a much faster deterioration of the workers' conditions than other causes of death. The only worker (8%) that died in the nest had been carried back into it by another worker 6 days before its death after having stayed outside for 6 days without ever interacting with its nestmates.

Finally, we exposed 70 young and presumably healthy workers (10 per colony) to 95% CO₂ to induce precocious aging [6, 7]. Of 52 workers that recovered after the treatment (4–9 per colony, median 8), 27 died within the following 2 weeks (2–7 per colony, 22%–100% of workers that recovered after CO₂ exposure, median 50%). Most dying CO₂-exposed workers (22 of 27, 81.5%) were observed leaving the nest 5 to 239 hr before their deaths (median 36 hr). All other dying

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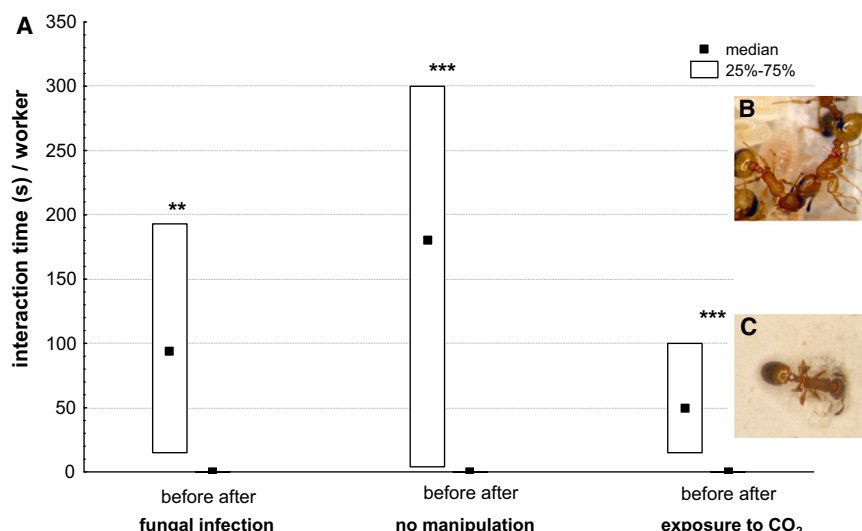


Figure 1. Duration of Social Interactions of Moribund Ants Before and After Leaving the Nest

Dying workers of the ant *Temnothorax unifasciatus* interacted for a significantly shorter time span with their nestmates after they had left their nests to die in isolation (A, duration of social interactions in seconds during 10 min before and 10 min after leaving, median and quartiles). Breaking off all social interactions occurred regardless of whether workers died of an infection with a pathogenic fungus, unknown causes in unmanipulated colonies, or prolonged exposure to 95% CO₂. This suggests that social withdrawal is not due to a behavioral manipulation by a parasite. (B) shows two healthy workers engaged in social feeding in the nest, and (C) shows a worker dying in isolation outside of the nest. Wilcoxon matched-pairs test, **p < 0.01, ***p < 0.0001.

ants were also found dead outside the nest, but, as above, their behavior before death was not monitored. The percentage of workers observed to die outside the nest was significantly larger than the proportion of time spent outside by healthy workers (70 control workers, 0–34% of scans observed outside, median 7.1%; χ^2 test, $\chi^2 = 93.13$, $p < 0.001$). Therefore, the finding that most workers die outside does not simply reflect the spatial and temporal distribution of live workers in the nest and the foraging arena. CO₂-exposed workers survived significantly longer when we prevented them from leaving the nest by closing its entrance (blocked, $n = 140$; open, $n = 52$; Cox-Mantel test, $I = 6.23$, $p < 0.0001$). This suggests that leaving the nest further decreases the life span of exposed ants and accelerates the process of dying.

Healthy nurses never left the nest during our observations, and healthy foragers regularly returned into it and moved outside only during some of the scans. For example, 23 foragers from the seven colonies with CO₂-exposed workers were observed outside the nest during 7% to 86% of the scans (median 14%). In contrast, dying workers continuously remained outside the nest during the last few days of their lives (22 dying CO₂-exposed workers, 100% of all scans, median test, $\chi^2 = 15.652$, $p < 0.001$). During this time, we never observed them foraging for food or water. Abandoning the nest generally resulted in the complete or almost complete stop of all active and passive contact with nestmates. In the case of parasite manipulation, social withdrawal would be expected only for fungus-infected ants. However, all dying workers broke off social contacts, regardless of whether they died from infection or other causes (Figure 1, duration of social interactions of moribund workers during 10 min before versus 10 min after leaving; Wilcoxon matched-pairs test: infection, $n = 31$, $z = 4.68$, $p < 0.0001$; unmanipulated colonies: $n = 12$, $z = 2.75$, $p < 0.01$; CO₂ exposition: $n = 22$, $z = 0.00$, $p < 0.0001$).

Before leaving the nest, infected workers and moribund workers in unmanipulated colonies engaged in active or passive interactions with their nestmates at a similar rate as control workers (permutation tests; infection: 59 control, 31 dying workers, $p = 0.130$; unmanipulated colonies: 499 control, 13 dying workers, $p = 0.108$). Workers dying from CO₂ exposure were considerably less active (59 control, 22 dying

workers, $p = 0.004$). Moribund workers appeared to leave their nests on their own accord, and we never observed them being attacked or carried out of the nest by other workers (with the exception of one dying worker, which we had returned into the nest). Dying workers did not receive any special treatment by their nestmates, even when infected with *Metarhizium* spores, in contrast to what is known for diseased individuals in other species [15, 16]. Five workers dying from unknown causes, which we gently placed back into the nest when they were no longer capable of walking, were first groomed by their nestmates and later left alone in the nest, where they eventually died. Their corpses were removed only 1 or 2 days after their deaths. In contrast, five of six moribund workers still capable of walking left the nest again within 18 hr after having been returned into it (median 40 min, range 2 s–18 hr), and only one worker was carried out by a nestmate after being groomed intensively for 130 min.

Taken together, our experiments document that moribund workers actively leave their nests and separate themselves from their nestmates. They die in solitude instead of seeking the company of their nestmates and attempting to restore their health in the safety of the nest. Because of the low probability of encountering nestmates away from the nest, abandoning the colony results in a complete or almost complete social isolation of the dying worker. *Metarhizium*-infected workers become contagious only a few days after their deaths when new conidia are formed. However, as a general response to approaching death, breaking off all social contacts and dying in isolation would greatly decrease the spread of diseases that are transmitted more quickly from individual to individual within the same nest.

Social withdrawal might thus be a simple mechanism through which infected individuals prevent the transmission of diseases to adult nestmates and the brood and thus reduce potential losses in their own inclusive fitness. It complements the fascinating group-level adaptations that enable social insects to cope with pathogen and parasite pressure such as mutual grooming, minimizing the contact to group members that handle potentially infectious garbage or feces, and walling off infected individuals [3, 12, 17, 18]. Leaving the nest before death may be particularly adaptive in species with very small societies and simple nests. Species like *T. unifasciatus* probably cannot afford putting infected workers under quarantine

in separate compartments or exposing specialized undertaker workers to contagious garbage or corpses [19, 20]. However, scattered reports about dying bees, wasps, and ants leaving their nests, including the mysterious disappearance of adult bees from their hives associated with colony collapse disorder [21], indicate that social withdrawal is not restricted to *Temnothorax* but is a more widespread phenomenon. In some cases, it may result from behavioral manipulation and may serve the interest of a parasite or pathogen [11–14, 22]. However, our observations of CO₂-exposed workers indicate that it also occurs in the absence of manipulating agents, when workers might perceive themselves as dying from an infection. Furthermore, the observation of dying-but-still-mobile workers fleeing from the nest after having been returned into it indicates that death in isolation is not merely an epiphenomenon of impaired maneuverability or loss of orientation. Instead, it appears to be an active and, in most cases, adaptive response of the dying ant to its own condition.

CO₂-exposed workers survived longer when prevented from leaving the nest. This suggests that leaving the nest further decreases the life expectancy of dying workers, probably because of desiccation in the foraging arena. Thus, dying in solitude resembles the suicidal sting of the honeybee worker [8] or the closing of the nest from outside by workers of *Forelius* ants [23] and constitutes a striking novel example of altruism in social animals that deserves additional attention.

Experimental Procedures

Study Organism and Laboratory Culture

We collected complete colonies of *T. unifasciatus* (Latreille, 1798) from their nests in stonewalls in a population at Gargnano, Lago di Garda (Italy) and transferred them into 10 × 10 × 3 cm³ plastic boxes with a plaster floor and a 50 × 12 × 3 mm³ cavity between two microscope slides serving as a nest site. Ants were provided with water and food (honey and pieces of fresh cockroaches) ad libitum and reared at 12/12 hr day/night cycles with temperatures of 25°C/20°C, respectively. All workers used in the experiments were individually marked with 30 µm thin copper wires (courtesy of Elektrisola) knotted around their petiole and postpetiole. We made sure that the markings did not hinder workers' movements but at the same time were impossible to remove.

Behavioral Observations

The duration of interactions among ants (antennation, exchange of liquid food, aggression, grooming, active participation in brood care, and carrying brood or adults) was observed with an accuracy of 1 s.

To document the behavior of workers that died spontaneously in unmanipulated colonies, we observed 28 laboratory colonies (15–100 workers per colony, median 40, total 1609) in ten 1 min scans per day for 5 days per week over 4 weeks and three times for 10 min per day for 6 days per week for 2 more weeks (i.e., 560 min per colony). In addition, dying workers (both dying inside and outside the nest) were observed for seven more scans. During the observation period, one single worker died in each of 13 colonies.

The behavior of workers dying from fungal infection was observed in seven experimental colonies each with a single queen, brood, and 20 individually marked workers of unknown age. We recorded both the active behavior and the behavior focal ants received from their nestmates during seven 1 min scans and three 10 min scans per day during 10 days in 2 weeks (i.e., 370 min per colony). Thereafter, ten randomly chosen workers per colony were infected (see below), while the other ten workers served as a control. Two hours after the treatment, we returned infected and control workers into their nests. After allowing the ants to adjust to the colony for 1 hr, we resumed the observations as above during the following 10 days (i.e., 370 min per colony). In addition, we monitored the mortality of workers until the twenty-first day after infection.

To observe the behavior of workers that died because of treatment with CO₂, we established seven colonies, each with a queen, brood, and 20 workers. We randomly chose 10 workers per colony and exposed them to 95% CO₂ for 80 hr, while the other 10 workers per colony served as a control. Workers that quickly recovered after the treatment were returned

into their nests, and the behavior of these workers was observed for 10 min per day in 1 min scans for 2 weeks (i.e., 140 min per colony).

In a parallel experiment, we forced workers to remain in the nest by blocking its entrance with a paper plug. In each of seven additional colonies, we exposed 20 randomly collected workers to 95% CO₂ for 80 hr, while 20 workers remained as a control. Exposed and control workers were kept separately in closed nests in groups of 20 workers each, and food was provided ad libitum directly into the nest. The survival of the ants was checked every day for 2 weeks and compared with the survival of ants that were allowed to leave the nest.

Infection with Fungal Spores

The generalist entomopathogenic fungus *M. anisopliae* var. *anisopliae* [24] (strain Ma275, obtained from S. Cremer and S. Tragust, Regensburg University) was cultivated on 6.5% Sabouraud dextrose agar plates at 23°C [15, 25]. For the infection of ants, we created a spore (conidia) suspension from a recently sporulating culture plate in a 0.05% solution of Triton X-100. The suspension was quantified with a hemocytometer and diluted to a concentration of 10⁹ spores/ml. Before infecting the ants, we determined the germination capacity of spores by spreading 100 µl of the suspension on a selective medium agar plate (containing dodecane, chloramphenicol, and streptomycin sulfate) and incubating for 18 hr at 23°C [25]. Germination rate was 99%. Quantities of 0.3 µl of the suspension were applied onto the cuticle of individual ants with a pipette [15, 26]. Preliminary tests revealed that >70% ants treated with the suspension died within a week after treatment (three colonies each with 10 infected and 10 control workers). Control ants received the same amount of 0.05% Triton X solution without any spores. Preliminary tests showed that the application of 0.3 µl Triton X solution did not affect the survival rate as compared to untreated ants, which is in accordance with results from previous studies [15]. To confirm that the death of infected and control individuals was indeed caused by *Metarhizium*, we sterilized dead workers [25] and transferred them into sterilized, closed Petri dishes. First hyphae were observed 12 hr after incubation and first spores (or spore clusters) 2 to 4 days later. *Metarhizium* spores, identified by shape, were found on 44 of 52 dead, infected workers. No spores were found on the bodies of five control workers that died during the experiment.

Statistical Analyses

Data were analyzed with Statistica version 6 and PAST 1.75b [27]. We compared the duration of interactions involving a moribund worker during the last 10 min of observation before leaving and 10 min observation after leaving by Wilcoxon matched-pairs test (Figure 1). Though the mortality of workers treated with spores or exposed to CO₂ differed among colonies, the behavior of dying workers did not vary among colonies (number of scans between dying workers leaving the nest and death: infected workers, median test, $\chi^2 = 4.516$, $p = 0.607$; CO₂-exposed workers, $\chi^2 = 7.377$, $p = 0.287$). We therefore pooled observations from different colonies. The decrease of social interactions involving moribund workers might in part be due to the lack of opportunity of interacting outside the nest. In principle, interaction frequency therefore needs to be corrected for the likelihood of encountering another ant, which cannot easily be estimated. Because moribund workers did not interact actively when they were antennated by a forager or another ant outside the nest nor when we returned them into the nest, we feel that we can safely conclude that the low social activity of moribund ants is caused not only by missed opportunity. The duration of social interactions of control ants and moribund ants before the latter left the nest was compared by permutation tests (10,000 permutations). To compare life spans, we used Cox-Mantel test because of the large number of censored life span data from control workers, of which most survived the complete duration of the experiment.

Acknowledgments

We thank S. Cremer, J. Korb, J. Seal, J.J. Boomsma, O. Rueppell, and four anonymous reviewers for helpful comments on the manuscript, as well as S. Tragust, E. Brunner, A. Wilhelm, A. Moesch, and S. Färnstein for technical assistance. B.W. was supported by a Deutscher Akademischer Austausch Dienst stipend (A/06/07501). Supported by Deutsche Forschungsgemeinschaft (He 1623/17).

Received: October 12, 2009

Revised: December 2, 2009

Accepted: December 2, 2009

Published online: January 28, 2010

References

- Hamilton, W.D. (1980). Sex versus non-sex versus parasite. *Oikos* 35, 282–290.
- Kraus, B., and Page, R.E., Jr. (1998). Parasites, pathogens, and polyandry in social insects. *Am. Nat.* 151, 383–391.
- Schmid-Hempel, P. (1998). *Parasites in Social Insects* (Princeton, NJ: Princeton University Press).
- Hamilton, W.D. (1987). Kinship, recognition, disease, and intelligence: Constraints of social evolution. In *Animal Societies: Theories and Facts*, Y. Itô, J.L. Brown, and J. Kikkawa, eds. (Tokyo: Japan Scientific Societies Press), pp. 81–102.
- Reymont, W.S. (1926). *Die polnischen Bauern. Roman in vier Jahreszeiten* (Jena: Eugen Diederichs Verlag).
- Moroń, D., Witek, M., and Woyciechowski, M. (2008). Division of labour among workers with different life expectancy in the ant *Myrmica scabrinodis*. *Anim. Behav.* 75, 345–350.
- Woyciechowski, M., and Moroń, D. (2009). Life expectancy and onset of foraging in the honeybee (*Apis mellifera*). *Insectes Soc.* 56, 193–201.
- Wilson, E.O. (1975). *Sociobiology* (Cambridge, MA: Harvard University Press).
- Wilson, E.O., Durlach, N.I., and Roth, L.M. (1958). Chemical releasers of necrophoric behavior in ants. *Psyche* 65, 108–114.
- Hölldobler, B., and Wilson, E.O. (1990). *The Ants* (Cambridge, MA: Harvard University Press).
- Hughes, D.P., Kathirithamby, J., Turillazzi, S., and Beani, L. (2004). Social wasps desert the colony and aggregate outside if parasitized: Parasite manipulation? *Behav. Ecol.* 15, 1037–1043.
- Oi, D.H., and Pereira, R.M. (1993). Ant behavior and microbial pathogens (Hymenoptera: Formicidae). *Fla. Entomol.* 76, 63–74.
- Durrer, S., and Schmid-Hempel, P. (1994). Shared use of flowers leads to horizontal pathogen transmission. *Proc. R. Soc. Lond. B. Biol. Sci.* 258, 299–302.
- Libersat, F., Delago, A., and Gal, R. (2009). Manipulation of host behavior by parasitic insects and insect parasites. *Annu. Rev. Entomol.* 54, 189–207.
- Ugelvig, L.V., and Cremer, S. (2007). Social prophylaxis: group interaction promotes collective immunity in ant colonies. *Curr. Biol.* 17, 1967–1971.
- Aubert, A., and Richard, F.-J. (2008). Social management of LPS-induced inflammation in *Formica polyctena* ants. *Brain Behav. Immun.* 22, 833–837.
- Cremer, S., Armitage, S.A., and Schmid-Hempel, P. (2007). Social immunity. *Curr. Biol.* 17, R693–R702.
- Wilson-Rich, N., Spivak, M., Fefferman, N.H., and Starks, P.T. (2009). Genetic, individual, and group facilitation of disease resistance in insect societies. *Annu. Rev. Entomol.* 54, 405–423.
- Julian, G.E., and Cahan, S. (1999). Undertaking specialization in the desert leaf-cutter ant *Acromyrmex versicolor*. *Anim. Behav.* 58, 437–442.
- Hart, A.G., and Ratnieks, F.L.W. (2001). Task partitioning, division of labour and nest compartmentalisation collectively isolate hazardous waste in the leafcutting ant *Atta cephalotes*. *Behav. Ecol. Sociobiol.* 49, 387–392.
- Vanengelsdorp, D., Evans, J.D., Saegerman, C., Mullin, C., Haubruge, E., Nguyen, B.K., Frazier, M., Frazier, J., Cox-Foster, D., Chen, Y., et al. (2009). Colony collapse disorder: a descriptive study. *PLoS ONE* 4, e6481.
- Andersen, S.B., Gerritsma, S., Yusa, K.M., Mayntz, D., Hywel-Jones, N.L., Billen, J., Boomsma, J.J., and Hughes, D.P. (2009). The life of a dead ant: the expression of an adaptive extended phenotype. *Am. Nat.* 174, 424–433.
- Tofilski, A., Couvillon, M.J., Evison, S.E.F., Helanterä, H., Robinson, E.J.H., and Ratnieks, F.L.W. (2008). Preemptive defensive self-sacrifice by ant workers. *Am. Nat.* 172, E239–E243.
- Vargo, E.L., and Blackwell, M., eds. (2005). *Insect-Fungal Associations: Ecology and Evolution* (New York: Oxford University Press).
- Lacey, L.A., and Brooks, W.M. (1997). Initial handling and diagnosis of diseased insects. In *Manual of Techniques in Insect Pathology*, L.A. Lacey, ed. (London: Academic Press), pp. 1–16.
- Hughes, W.O.H., Eilenberg, J., and Boomsma, J.J. (2002). Trade-offs in group living: Transmission and disease resistance in leaf-cutting ants. *Proc. Biol. Sci.* 269, 1811–1819.
- Hammer, Ø., Harper, D.A.T., and Ryan, P.D. (2001). PAST: Paleontological statistics software package for education and data analysis. *Palaeont. Elect.* 4, 1–9.