



Sanitary behavior in queenright and queenless ant colonies

Julia Giehr*, Tomer J. Czaczkes, Jürgen Heinze

University of Regensburg, Department of Evolutionary Biology/Zoology, Universitätsstr. 31, D-93053, Regensburg, Germany



ARTICLE INFO

Keywords:

Defecation
Hygienic behavior
Temnothorax
Ants
Sanitary behavior

ABSTRACT

Waste disposal is important for maintaining the health of animal societies. Adults and offspring produce large amounts of waste and feces that could contain pathogens or toxins and may need to be stored away from the young or adult individuals. In social insects, the worker caste is responsible for nest maintenance, including sanitary behavior, and waste disposal strategies vary between species. However, individual task allocation is generally affected by queen presence and worker efficiency often decreases in the absence of a queen.

Here we show that most (74%) colonies of the cavity-dwelling ant *Temnothorax crassispinus* construct up to two localized indoor 'latrines', which are used for defecation and only very rarely also as waste dumps. Restriction of defecation to designated areas affects the growth of mold inside the nest. Defecation strategies of colonies are furthermore affected by queen presence, with workers from queenless nests more frequently defecating outside the nest and forming latrines. As colonies do not actively avoid moldy nests, mold seems to not necessarily be a threat to the colony. While solid waste management has been more extensively studied in social insects, this study contributes a rare insight into the organization of non-easily transportable fecal waste.

1. Introduction

The societies of ants, bees, and wasps are generally characterized by division of labor between reproductives and non-reproductive workers. The loss of the reproductive individual(s) typically leads to tremendous changes in colony organization. In particular, in species with small societies, non-reproductives may begin to compete aggressively to obtain direct fitness by producing males from unfertilized eggs (Cole, 1981; Franks and Scovell, 1983; Heinze et al., 1997; Stroeymeyt et al., 2007) or, in a few taxa, even by mating and producing both male and female offspring (Monnin et al., 2002; Peeters and Tsuji, 1993).

Queen loss and the take-over of reproduction by workers affects both individual worker physiology and the structure of the whole society. On an individual level, queen loss affects brain dopamine titers (Harris and Woodring, 1995; Cuvillier-Hot and Lenoir, 2006; Shimoji et al., 2017), learning capability (Evans et al., 2016), and life span (Dixon et al., 2014; Hartmann and Heinze, 2003; Tsuji et al., 1996). On a societal level, queen loss may weaken nestmate discrimination, which in turn facilitates colony usurpation by alien conspecific queens, or by social parasites (Buschinger, 2009; Chapman et al., 2009; Tschinkel, 1996; Van Oystaeyen et al., 2013). Furthermore, queen loss may lower overall activity levels and change collective behavior (Berton et al., 1992; Gobin et al., 2003; Jaycox, 1970; Reeve and Gamboa, 1987; Sumana and Starks, 2004). For example, workers from queenless

colonies of the ant *Temnothorax curvispinosus* interact less with the brood and retrieve misplaced larvae more slowly than workers from queenright colonies (Keiser et al., 2018).

More importantly, queenless colonies appear to be less resistant to fungal infections, suggesting that the presence of a queen may affect collective immune responses, and thus the spread of diseases (Keiser et al., 2018). Waste, corpses, and feces may contain infectious agents and therefore are potentially dangerous (Currie et al., 1999; Hart and Ratnieks, 2002; Waddington and Hughes, 2010; Weiss, 2006). Nest hygiene and disease prevention are thus important aspects of the collective behavior of social insects. Many social animals have therefore evolved efficient waste management, including external or internal dumps and latrines (Bernadou et al., 2018; Bot et al., 2001; Czaczkes et al., 2015; Eastwood, 1997; Farji-Brener and Medina, 2000; Hölldobler and Wilson, 1977, 1978; Jackson and Hart, 2009; O'Neal and Markin, 1973; Peeters et al., 1994; Waddington and Hughes, 2010).

We found that 27% of *T. crassispinus* colonies are queenless under natural conditions (unpublished results). As queen loss affects worker behavior (Berton et al., 1992; Gobin et al., 2003; Jaycox, 1970; Reeve and Gamboa, 1987; Sumana and Starks, 2004) and reduces colony resistance to infection in *T. curvispinosus* (Keiser et al., 2018), we asked whether queen loss might affect defecation and sanitary behavior in the related ant *T. crassispinus*. We find that defecation behavior and waste management varies among colonies and also differs between queenright

* Corresponding author.

E-mail addresses: Julia.Giehr@ur.de (J. Giehr), Tomer.Czaczkes@ur.de (T.J. Czaczkes), Juergen.Heinze@ur.de (J. Heinze).

and queenless colony fragments. Our study shows that latrine formation and also queen presence affect microbiota growth inside the colonies, but that ant colonies do not avoid moldy nests. These findings can provide a basis for further studies on sanitary behavior and the co-existence with microbiota in ant colonies.

2. Material and methods

Temnothorax crassispinus is a small (2–3 mm), monogynous (single-queen) ant species that lives in small colonies with 20–300 workers in acorns or twigs. In summer, many natural nests are queenless (27%, unpublished data), as individual colonies may seasonally inhabit multiple nest sites (seasonal polydomy, Strätz & Heinze, 2004). We collected 29 queenright colonies near Regensburg, Germany, and split each into a queenright and queenless half, resulting in a total of 58 colony fragments. These fragments were then standardized to consist of 25 workers each (with or without queen). We added 25 larvae to all queenright and 10 of 29 queenless colonies, the remaining 19 queenless colonies did not receive larvae, so as to investigate the effects of queen-produced larvae.

Colonies were housed in small three-chambered plastic boxes (10 × 10 × 3 cm³; one chamber each for nest, food, and water) with a moistened plaster floor under 12 h 26 °C/ 12 h 22 °C day/night cycles). The nest was composed of the area surrounded by a plastic frame (554 mm²) sandwiched between two microscope slides (1.2 × 5 × 0.3 cm³) with a narrow entrance (0.3 × 1 × 0.3 cm³). It was covered with an opaque film to ensure that the nest cavity was dark.

Colonies were fed twice per week with fruit flies (*Drosophila melanogaster*) and honey. Honey was colored with commercially available food dyes (red: Allura red AC, E129, 12.5% pure color, 2% aluminum; blue: Brilliant blue FCF, E133, 9.26% pure color, 3.6% aluminum, carrying agent sulfate / chloride, RBV Birkmann GmbH & Co; 4 g per liter of solution). Red and blue food coloring was used for queenright and queenless colonies, respectively, for convenience, as in previous experiments the color of food dyes was found to have no influence on defecation behavior (Bernadou et al., 2018; Czaczkes et al., 2015).

Four weeks after colony establishment we took digital photographs of the nest box and nest, which were analyzed blindly. At this time 0–4 (median, Q1, Q3: 1, 0, 1) workers had died or vanished per colony. We then noted the presence and number of latrines within each colony. Accumulations of defecation spots were defined as latrines when spots had the color of the food and were concentrated in an area smaller than 1/3 of the nest (see Fig. 1). Randomly distributed spots were not counted as latrines (see photos in Supplement S2 & S3). Nine queenright and two queenless colonies had to be excluded from the latrine analysis due to excessive mold growth, which prevented an accurate determination of defecation spots. The area covered by latrines and mold, and the distance of the latrines from the nest entrance, and the distance of latrines from brood piles, were measured using ImageJ. Four weeks after the photographs were taken we put 17 related queenless and queenright split-fragments into a new, clean arena (diameter: 14 cm) to allow them to reunite and to observe nest choice relative to queen presence and mold growth. The remaining 12 queenless and queenright fragment were chosen randomly and kept separated for further, independent experiments.

Data were analyzed with R v. 3.2.3 software (R Development Core Team, 2008) with packages “ggplot2” (Wickham, 2009) for the plots and “vegan” (Oksanen et al., 2017) for conducting the PerMANOVA as data were non-parametric in the case of count data or not normally distributed (Shapiro-Wilk normality test: $p < 0.05$). PerMANOVA was used to test the effects of queen presence, larvae presence, latrine presence and outside defecation on the defecation behavior of the colonies. In the results, factors are given in the order they were included in the test. Count data were compared using Chi-square test (χ^2) and pairwise comparisons were conducted using Mann-Whitney U test (“Wilcoxon rank sum test with continuity correction”) followed by a

correction for a false discovery rate (“fdr”) (Benjamini and Hochberg, 1995). We grouped queenless colonies with and without larvae for analyses, as we did not find any effect of larva presence in queenless colonies. They did not differ in the frequencies of latrine formation (with larvae: 6/19; without larvae 1/8, $\chi^2 = 1.36$, $p = 0.243$; one colony could not be evaluated due to excessive mold growth), defecation outside (with larvae: 13/19; without larvae: 8/10; $\chi^2 = 0.439$, $p = 0.507$), nor latrine size ($W = 27$, $p = 0.799$), or mold distribution ($W = 99$, $p = 0.865$).

3. Results

In almost all colonies (89%, 42/47), the ants defecated inside the nest, and 74% (31/42 colonies) of the colonies with defecation in the nest showed one or two well-defined latrine patches (Fig. 1). In total, 40 latrine patches were formed, mostly along the corners of the nest (37/40 latrines) with distance to the nest entrance (median, Q1, Q3: queenless: 21.15, 9.45 mm, 48.81 mm; queenright: 16.89 mm, 10.92 mm, 29.14 mm; $W = 140$, $p = 0.421$). Queenless and queenright colonies did not differ in the presence ($\chi^2 = 0.260$, $df = 1$, $p = 0.6125$) or number of latrines ($\chi^2 = 0.816$, $df = 2$, $p = 0.665$). Furthermore, comparing the presence of latrines between the queenright and queenless halves of the same colony did not reveal any colony-specific trend (Spearman’s rank correlation, $n = 20$, $rS = -0.132$, $p = 0.578$).

Latrines made up to 26% (150.67 mm²) of the inner nest (area median, Q1, Q3: queenless: 26.18 mm², 19.01 mm², 41.21 mm²; queenright: 23.37 mm², 18.16 mm², 62.33 mm²; $W = 118$, $p = 0.984$). Only a minority of latrines (queenless: 2 of 21, 9.5%; queenright: 2/19 latrines, 10.5%) contained other refuse particles (one piece of *Drosophila* located in one latrine each in two queenright colonies and two queenless colonies) both in queenless and queenright colonies, and none of the latrines contained dead ants, i.e., latrines did not serve as general waste dumps. Only 21% (14/58) of all colonies stored food items (fresh pieces of *Drosophila*) inside the colony, but never in a centralized place. Rather, food items were widely distributed throughout the nest (indicated by the yellow circles in supplements S2 & S3).

Brood piles were always kept away from the latrines (distance median, Q1, Q3: queenless: 30.93 mm, 26.20 mm, 41.88 mm; queenright: 29.12 mm, 23.74 mm, 34.53 mm) and non-centralized defecation areas. Only in two queenright colonies were single larvae found on defecation spots. In most queenless colonies (21/29) workers also defecated outside the nest, especially under the nest, while external defecation was significantly less common in queenright colonies (11/29; $\chi^2 = 6.97$, $df = 1$, $p = 0.0017$, see figure S1). Workers from colonies with well-defined latrines inside the nest defecated more frequently outside the nest (25/31) than workers from colonies with random defecation spots inside the nest (4/11) ($\chi^2 = 7.5$, $p = 0.006$; PerMANOVA: defecation outside the nest: queen presence $F = 1.1$, $df = 1$, $p = 0.22$, presence of larvae $F = 2.8$, $df = 1$, $p = 0.1$, latrine presence inside the nest $F = 8.4$, $df = 1$, $p = 0.0052$). When defecating outside, workers from queenright colonies were more likely to form localized latrines under the nest than workers from queenless colonies (5/21 queenless colonies, 9/11 queenright colonies; $\chi^2 = 9.9$, $df = 1$, $p = 0.002$) (PerMANOVA latrine formation outside the nest: queen presence $F = 14.6$, $df = 1$, $p = 0.0005$, presence of larvae $F = 0.75$, $df = 1$, $p = 0.39$, latrine presence inside the nest $F = 0.55$, $df = 1$, $p = 0.63$).

Under laboratory conditions, *T. crassispinus* nests were quickly covered by mold (area median, Q1, Q3: 44.37 mm², 0.00 mm², 403.95 mm²) and the degree of microbiota growth inside the nest appeared to be linked to the presence of the queen and the individuals’ defecation behavior (PerMANOVA: queen presence: $F = 3.71$, $df = 1$, $p = 0.041$, larvae presence: $F = 1.27$, $df = 1$, $p = 0.29$, latrine presence: $F = 13.61$, $df = 4$, $p = 0.001$, defecation outside the nest: $F = 1.37$, $df = 1$, $p = 0.27$, see Fig. 2). Colonies were less moldy when workers

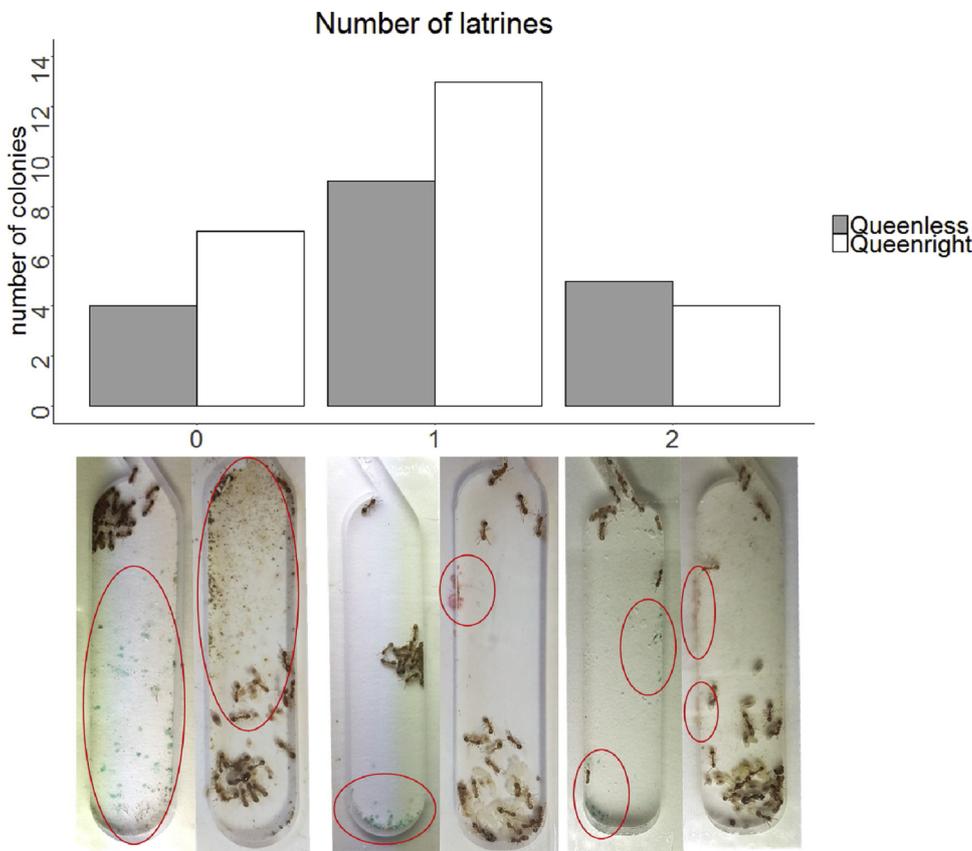


Fig. 1. Number of colonies without (0) with one (1) or with two (2) locally defined latrines in queenless (black) and queenright (grey) *Temnothorax crassispinus* nests. Defecation and latrine areas are marked with a red circle. In the two leftmost nests, defecation spots are randomly distributed throughout large parts of the nest and not locally concentrated in a latrine area.

defecated outside (median, Q1, Q3: 0.00 mm², 0.00 mm², 45.75mm²) than colonies with only inside defecation (median, Q1, Q3: 450.57mm², 83.95mm², 563.06mm²; $W = 704$, $p < 0.0001$). Furthermore, the formation of localized latrines inside the nest (median, Q1, Q3: 14.28 mm², 0.00 mm², 47.21 mm²) appeared to reduce mold growth more than random defecation (median, Q1, Q3: 291.71 mm², 122.41 mm², 473.33 mm²; $W = 300.5$, $p = 0.00014$) and the area covered by latrines and mold growth were positively correlated (Spearman’s rank correlation: $rS = 0.45$, $p = 0.01$).

When we allowed 17 split colonies to reunite four weeks later, eight colony fragments moved in with their nestmates in the more or equally moldy nest, five colonies merged in the less moldy nest and in four cases colonies did not reunite at all. Interestingly, regarding the colonies that

moved, six left their clean, mold-free nests to move into nests with considerable microbiota growth. This indicates that the ants do not avoid moldy nests.

4. Discussion

In most (89%) studied colonies of the ant *T. crassispinus*, individuals defecated inside the nest, and in most nests (74%) they defecated only in particular areas. These areas appear to be defecation sites (latrines or toilets c.f. Czaczkes et al., 2015), as their color matched the artificially colored food. In contrast to waste disposal sites or middens (Peeters et al., 1994; Rettenmeyer, 1963; Wilson and Brown, 2005), only two of 40 latrines contained left-over food items or other waste. *T. crassispinus*

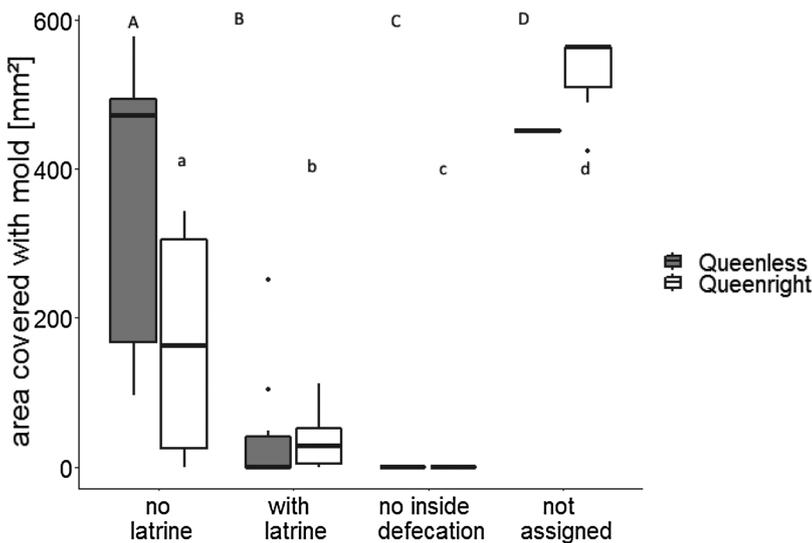


Fig. 2. Area of the inner nest covered by mold in relation to the individuals’ defecation behavior and queen presence. In colonies without latrines (“no latrines”) the inner nest is covered up to 100% by mold, but the formation of latrines inside the nest (“with latrines”) significantly reduces mold growth. The avoidance of inside defecation (“no inside defecation”) can stop mold growth completely. In a few colonies, mold growth was so intense that they could not be assigned (“not assigned”) to any of the other groups. Boxplots show median, 25 and 75 quartiles, and 95% percentiles. *Post hoc* comparisons among groups significantly different at $p = 0.05$, adjusted following Benjamini-Hochberg (1995), are displayed by different letters (lower case for queenright colonies, capitals for queenless colonies). Mold growth does not differ significantly between queenless and queenright groups.

rarely store large amounts of food in their nests and the random distribution of the few scattered food residues indicated that they do not deposit left-over food items in specific sites. Instead, workers were seen removing decaying food from the nest (unpublished observations).

Many ant species have waste disposal areas, where they put droplets of feces and other waste products (Bernadou et al., 2018; Hart and Ratnieks, 2001; Hölldobler and Wilson, 1978; Weiss, 2006). However, the formation of latrines inside ant nests has so far only been reported for a few formicine ants (e.g. Hölldobler 1975, including *Lasius niger* (Czaczkes et al., 2015), the ponerine ant *Platythyrea punctata* (Bernadou et al., 2018) and *Crematogaster smithi*, (S. Cremer & J. Oettler, unpublished)). Feces and other excretions can promote pathogen growth and thus pose a threat to insect societies (Copley et al., 2012; Hart and Ratnieks, 2001). Together with other types of pathogen defense (e.g., Cremer et al., 2007), defecation in well-defined areas might minimize infection risk. While foragers may easily defecate outside the nest (Bernadou et al., 2018), the presence of indoor latrines may protect younger workers and the queen from predation and other external hazards (Czaczkes et al., 2015).

In *T. crassispinus*, the formation of localized latrines appears to reduce mold growth. Natural colonies of *T. crassispinus* live in relatively ephemeral sites, such rotting twigs, under bark, and in hollow acorns, and mold prevention might increase the stability of the nest material. The resting workers avoided moldy patches inside the nest, and brood items were kept away from them (see also Karlik et al., 2016). Nevertheless, single individuals themselves appeared not to be strongly affected by mold. When we allowed colony fragments to reunite at the end of the experiment, some of them moved into the moldier nest. Behavioral observations revealed that active workers frequently handled the mold, relocating it, removing fragments or using pieces of mold to close the nest entrance (unpublished data). Furthermore, colonies remained in a moldy nests rather than moving into new nest sites. These observations are consistent with previous studies, which show that mature ant colonies (*Myrmica rubra*, Leclerc et al., 2018; *Monomorium pharaonis*, Pontieri et al., 2014) or founding queens (*Formica selysi*, Bruetsch et al., 2014) often actively choose pathogen-contaminated substrate for nest foundation. These studies conclude that intentional pathogen contact might lead to immunization (Brütsch et al., 2014; Leclerc et al., 2018; Pontieri et al., 2014).

Colonies differed in sanitary behavior, but why workers in a minority of colonies defecated randomly throughout the nest or only outside remains unclear. Variation in hygienic behavior in honey bees has been suggested to have a genetic basis (Oxley et al., 2010; Rothenbuhler, 1964), but in our study latrine formation was not significantly correlated between queenless and queenright fragments of the same colony. Instead, differences appeared to be in part associated with presence or absence of the queen: workers from queenless colonies defecated more frequently outside the nest than workers from queenright colonies. We can only speculate as to why queen loss results in a slightly changed defecation behavior. While workers refrain from laying large numbers of eggs in the presence of the queen, several socially dominant workers oviposit in queenless colonies (El-Shehaby et al., 2012) and the number of brood items is often larger than in queenright colonies of the same size (unpublished data). This means that more food needs to be retrieved, and, as in *Platythyrea punctata* (Bernadou et al., 2018), increased foraging activity might be associated with more outside defecation.

Nevertheless, the occurrence of latrines inside the colony documents sanitary behavior in *Temnothorax* ants and the capability of workers to form well-defined latrine patches. The connection between latrines and mold growth inside the nest might indicate that these products and the consequent growth of microbiota might not be harmful for the colony, and may indeed be beneficial. Indeed, Varoudis et al. (2018) showed that frass and plant tissue play an important role in structuring natural nest sites of this species.

5. Conclusion

Our study reveals that sanitary behavior, as in our case latrine formation, is not only of importance in highly structured social systems as honey bees (Rothenbuhler, 1964) or *Lasius niger* ant colonies (Czaczkes et al., 2015), but can also be found in less complex societies, such as the small *Temnothorax* colonies. Localized latrines seem to improve nest cleanliness and reduce mold growth in *Temnothorax* ants. In contrast to solid waste management, studies on defecation behavior in social insects are rare, and further studies are needed to understand the function of latrines, their localization, and their role in microbiota growth.

Declaration of interest

The author(s) declare(s) that they have no competing interests.

Funding

This study was supported by the Deutsche Forschungsgemeinschaft (He 1623/39). Funding agency had no role in study design, data collection, analysis and interpretation, or writing the manuscript.

Authors' contributions

JG performed the experiment, designed the study and analyzed the data; JG, TJC and JH wrote the manuscript and interpreted the data. All authors read and approved the final manuscript.

Acknowledgement

We thank Lisa Senninger and Katja Ruhland for their help to collect and maintain the colonies and Jennifer Wallner and Clara Hartmann for evaluating the photographs.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.beproc.2019.04.017>.

References

- Benjamini, Y., Hochberg, Y., 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J. R. Stat. Soc. Ser. B* 57, 289–300.
- Bernadou, A., Czaczkes, T., Heinze, J., 2018. From inside to outside and back again: changing waste dump formation, defecation and worker localization in a clonal ant. *Insectes Soc.* 65, 133–140. <https://doi.org/10.1007/s00040-017-0594-3>.
- Berton, F., Lenoir, A., Le Roux, G., Le Roux, A., 1992. Effect of orphaning on the effectiveness of queen attraction and on worker behavioral repertoire in *Cataglyphis cursor* (Hymenoptera: Formicidae). *Sociobiology* 21, 301–313.
- Bot, A.N., Currie, C.R., Hart, A.G., Boomsma, J.J., 2001. Waste management in leaf-cutting ants. *Ethol. Ecol. Evol.* 13, 225–237. <https://doi.org/10.1080/08927014.2001.9522772>.
- Bruetsch, T., Felden, A., Reber, A., Chapuisat, M., 2014. Ant queens (Hymenoptera: Formicidae) are attracted to fungal pathogens during the initial stage of colony founding. *Myrmecol. News* 20, 71–76.
- Buschinger, A., 2009. Social parasitism among ants: a review (Hymenoptera: Formicidae). *Myrmecol. News* 12, 219–235.
- Chapman, N.C., Nanork, P., Gloag, R.S., Wattanachaiyingcharoen, W., Beekman, M., Oldroyd, B.P., 2009. Queenless colonies of the Asian red dwarf honey bee (*Apis florea*) are infiltrated by workers from other queenless colonies. *Behav. Ecol.* 20, 817–820. <https://doi.org/10.1093/beheco/arp065>.
- Cole, B.J., 1981. Dominance hierarchies in *Leptothorax* ants. *Science* 212, 83–84.
- Copley, T.R., Giovenazzo, P., Jabaji, S.H., 2012. Detection of *Nosema Apis* and *N. ceranae* in honeybee bottom scraps and frass in naturally infected hives. *Apidologie* 43, 753–760. <https://doi.org/10.1007/s13592-012-0147-8>.
- Cremer, S., Armitage, S.A., Schmid-Hempel, P., 2007. Social immunity. *Curr. Biol.* 17, R693–R702. <https://doi.org/10.1016/j.cub.2007.06.008>.
- Currie, C.R., Mueller, U.G., Malloch, D., 1999. The agricultural pathology of ant fungus gardens. *Proc. Natl. Acad. Sci. U. S. A.* 96, 7998–8002. <https://doi.org/10.1073/pnas.96.14.7998>.
- Cuvillier-Hot, V., Lenoir, A., 2006. Biogenic amine levels, reproduction and social dominance in the queenless ant *Streblognathus peetersi*. *Naturwissenschaften* 93,

- 149–153.
- Czaczkas, T.J., Heinze, J., Ruther, J., 2015. Nest etiquette - where ants go when nature calls. *PLoS One* 10, e0118376. <https://doi.org/10.1371/journal.pone.0118376>.
- Dixon, L., Kuster, R., Rueppell, O., 2014. Reproduction, social behavior, and aging trajectories in honeybee workers. *Age* 36, 89–101. <https://doi.org/10.1007/s11357-013-9546-7>.
- Eastwood, R., 1997. Field observations on the symbiotic interactions of *Ogyris genoveva* (hewitson) and *Ogyris zosine* (hewitson) (Lepidoptera: Lycaenidae) with *Camponotus* spp. (Hymenoptera: Formicidae). *Austr. Entomol.* 24, 137.
- El-Shehaby, M., Abd-el Reheem, A., Heinze, J., 2012. Determinants of worker reproduction in queenless colonies of the ant *Temnothorax crassispinus* (Karavaiev, 1926) (Hymenoptera: Formicidae). *Myrmecol. News* 17, 21–26.
- Evans, L.J., Raine, N.E., Leadbeater, E., 2016. Reproductive environment affects learning performance in bumble bees. *Behav. Ecol. Sociobiol.* 70, 2053–2060. <https://doi.org/10.1007/s00265-016-2209-9>.
- Farji-Brener, A.G., Medina, C.A., 2000. The importance of where to dump the refuse: seed banks and fine roots in nests of the leaf-cutting ants *Atta cephalotes* and *A. colombica*. *Biotropica* 32, 120–126.
- Franks, N.R., Scovell, E., 1983. Dominance and reproductive success among slave-making worker ants. *Nature* 304, 724–725. <https://doi.org/10.1038/304724a0>.
- Gobin, B., Heinze, J., Strätz, M., Roces, F., 2003. The energetic cost of reproductive conflicts in the ant *Pachycondyla obscuricornis*. *J. Insect Physiol.* 49, 747–752. [https://doi.org/10.1016/S0022-1910\(03\)00111-2](https://doi.org/10.1016/S0022-1910(03)00111-2).
- Harris, J.W., Woodring, J., 1995. Elevated brain dopamine levels associated with ovary development in queenless worker honey bees (*Apis mellifera* L.). *Comp. Biochem. Physiol. C* 111, 271–279. [https://doi.org/10.1016/0742-8413\(95\)00048-S](https://doi.org/10.1016/0742-8413(95)00048-S).
- Hart, A.G., Ratnieks, F.L., 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. <https://doi.org/10.1007/s002650000312>.
- Hart, A.G., Ratnieks, F.L., 2002. Waste management in the leaf-cutting ant *Atta colombica*. *Behav. Ecol.* 13, 224–231. <https://doi.org/10.1093/beheco/13.2.224>.
- Hartmann, A., Heinze, J., 2003. Lay eggs, live longer: division of labor and life span in a clonal ant species. *Evolution* 57, 2424–2429. <https://doi.org/10.1111/j.0014-3820.2003.tb00254.x>.
- Heinze, J., Puchinger, W., Hölldobler, B., 1997. Worker reproduction and social hierarchies in *Leptothorax* ants. *Anim. Behav.* 54, 849–864.
- Hölldobler, B., Wilson, E.O., 1977. Colony-specific territorial pheromone in the african weaver ant *Oecophylla longinoda* (Latreille). *Proc. Natl. Acad. Sci. U. S. A.* 74, 2072–2075.
- Hölldobler, B., Wilson, E.O., 1978. The multiple recruitment systems of the african weaver ant *Oecophylla longinoda* (Latreille) (Hymenoptera: Formicidae). *Behav. Ecol. Sociobiol.* 3, 19–60. <https://doi.org/10.1007/BF00300045>.
- Jackson, D.E., Hart, A.G., 2009. Does sanitation facilitate sociality? *Anim. Behav.* 1, e1–e5.
- Jaycox, E.R., 1970. Honey bee queen pheromones and worker foraging behavior. *Ann. Entomol. Soc. Am.* 63, 222–228. <https://doi.org/10.1093/aesa/63.1.222>.
- Karlik, J., Epps, M.J., Dunn, R.R., Penick, C.A., 2016. Life inside an acorn: How microclimate and microbes influence nest organization in *Temnothorax* ants. *Ethology* 122, 790–797.
- Keiser, C.N., Vojvodic, S., Butler, I.O., Sartain, E., Rudolf, V.H.W., Saltz, J.B., 2018. Queen presence mediates the relationship between collective behaviour and disease susceptibility in ant colonies. *J. Anim. Ecol.* 87, 379–387. <https://doi.org/10.1111/1365-2656.12696>.
- Leclerc, J.B., Pinto Silva, J., Detrain, C., 2018. Impact of soil contamination on the growth and shape of ant nests. *R. Soc. Open Sci.* 5, 180267. <https://doi.org/10.1098/rsos.180267>.
- Monnin, T., Ratnieks, F.L.W., Jones, G.R., Beard, R., 2002. Pretender punishment induced by chemical signalling in a queenless ant. *Nature* 419, 61–65. <https://doi.org/10.1038/nature00932>.
- O'Neal, J., Markin, G., 1973. Brood nutrition and parental relationships of the imported fire ant *Solenopsis invicta*. *J. Ga. Entomol. Soc.* 8, 294–303.
- Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlenn, D., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Szoecs, E., Wagner, H., 2017. *Vegan: Community Ecology Package*. R Package Version 2.4-3.
- Oxley, P.R., Spivak, M., Oldroyd, B.P., 2010. Six quantitative trait loci influence task thresholds for hygienic behaviour in honeybees (*Apis mellifera*). *Mol. Ecol.* 19, 1452–1461.
- Peeters, C., Tsuji, K., 1993. Reproductive conflict among ant workers in *Diacamma* sp. from Japan: dominance and oviposition in the absence of the gamergate. *Insectes Soc.* 40, 119–136.
- Peeters, C., Hölldobler, B., Moffett, M., Ali, T.M.M., 1994. "wall-papery" and elaborate nest architecture in the ponerine ant *Harpegnathos saltator*. *Insectes Soc.* 41, 211–218. <https://doi.org/10.1007/BF01240479>.
- Pontieri, L., Vojvodic, S., Graham, R., Pedersen, J.S., Linksvayer, T.A., 2014. Ant colonies prefer infected over uninfected nest sites. *PLoS One* 9, e111961. <https://doi.org/10.1371/journal.pone.0111961>.
- R Development Core Team, 2008. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.
- Reeve, H.K., Gamboa, G.J., 1987. Queen regulation of worker foraging in paper wasps: a social feedback control system (*Polistes fuscatus*, Hymenoptera: Vespidae). *Behaviour* 102, 147–167.
- Rettenmeyer, C.W., 1963. Behavioral studies of army ants. *Univ. Kans. Sci. Bull.* 44, 281–465.
- Rothenbuhler, W.C., 1964. Behavior genetics of nest cleaning in honey bees. IV. responses of F1 and backcross generations to disease-killed brood. *Am. Zool.* 4, 111–123.
- Shimoi, H., Aonuma, H., Miura, T., Tsuji, K., Sasaki, K., Okada, Y., 2017. Queen contact and among-worker interactions dually suppress worker brain dopamine as a potential regulator of reproduction in an ant. *Behav. Ecol. Sociobiol.* 71, 35. <https://doi.org/10.1007/s00265-016-2263-3>.
- Stroeymeyt, N., Brunner, E., Heinze, J., 2007. "Selfish worker policing" controls reproduction in a *Temnothorax* ant. *Behav. Ecol. Sociobiol.* 61, 1449–1457. <https://doi.org/10.1007/s00265-007-0377-3>.
- Sumana, A., Starks, P.T., 2004. The function of dart behavior in the paper wasp, *Polistes fuscatus*. *Naturwissenschaften* 91, 220–223.
- Tschinkel, W., 1996. A newly-discovered mode of colony founding among fire ants. *Insectes Soc.* 43, 267–276.
- Tsuji, K., Nakata, K., Heinze, J., 1996. Lifespan and reproduction in a queenless ant. *Naturwissenschaften* 83, 577–578.
- Van Oystaeyen, A., Alves, D.A., Oliveira, R.C., do Nascimento, D.L., do Nascimento, F.S., Billen, J., Wenseleers, T., 2013. Sneaky queens in *Melipona* bees selectively detect and infiltrate queenless colonies. *Anim. Behav.* 86, 603–609.
- Varoudis, T., Swenson, A.G., Kirkton, S.D., Waters, J.S., 2018. Exploring nest structures of acorn dwelling ants with x-ray microtomography and surface-based three-dimensional visibility graph analysis. *Philos. Trans. R. Soc. B* 373, 20170237.
- Waddington, S.J., Hughes, W.O.H., 2010. Waste management in the leaf-cutting ant *Acromyrmex echinatior*: the role of worker size, age and plasticity. *Behav. Ecol. Sociobiol.* 64, 1219–1228. <https://doi.org/10.1007/s00265-010-0936-x>.
- Weiss, M.R., 2006. Defecation behavior and ecology of insects. *Annu. Rev. Entomol.* 51, 635–661. <https://doi.org/10.1146/annurev.ento.49.061802.123212>.
- Wickham, H., 2009. *ggplot2: Elegant Graphics for Data Analysis*. URL: Springer-Verlag, New York. <http://ggplot2.org>.
- Wilson, E.O., Brown, W.L., 2005. Behavior of the cryptobiotic predaceous *Anteurhopalothrix heliscata*, n. sp. (Hymenoptera: Formicidae: Basicerotini). *Insectes Soc.* 31, 408–428.