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Original Article

Larval chemical cues induce rapid changes in foraging preferences of ant workers (Hymenoptera: Formicidae)

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Abstract

In many animals that form cooperative social systems, such as ants, only a small number of individuals take over foraging duties. These specialized foragers not only have to fulfill their own nutritional needs but also the demands of non-foraging individuals residing in the nest. This raises the question of how foragers receive cues regarding nutritional requirements of their nestmates to adjust their foraging preferences. In ants, which like most insects primarily communicate chemically, larvae might convey their demands through the emission of odors. Using the acorn ant *Temnothorax longispinosus*, we first demonstrated that the presence of larvae increases protein foraging but not carbohydrate foraging. We exposed colonies lacking their own larvae to larval chemical extracts and found that larval odors rapidly increased protein foraging but did not alter carbohydrate foraging. Gas chromatography and mass spectrometry analyses revealed that larval extracts primarily consist of *n*-alkanes, with n-alkanes accounting for more than 90% of all larval CHCs. Carbohydrate and protein foraging appeared to be independent of each other, indicating separate regulatory mechanisms. Our study offers new insights into colony-homeostasis maintenance and emphasizes the vital role of larvae and larval chemical cues in regulating colony behavior.

Key words: Division of labor, brood, chemical communication, larvae, cuticular hydrocarbons, behavioral decisions.

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Introduction

Which nutrients an individual forages for is largely regulated by physiological demands. For example, *Drosophila melanogaster* fruit fly females switch from a carbohydrate-rich to a protein-rich diet after mating to facilitate the production of protein-rich oocytes (Ribeiro & Dickson 2010, Vargas & al. 2010, Münch & al. 2022). In many species in which cooperation, maternal care, and / or group living evolved, individual foraging decisions are furthermore linked to the social environment, for instance, when parents adjust foraging preferences to fulfill their offspring's nutritional requirements. Studying the mechanisms via which the social environment modulates individual foraging decisions is important to understand how social systems can respond to changes in nutritional demands and by that achieve homeostasis and ideal conditions for the development of the offspring.

An ideal system to achieve this are social hymenopterans, that is, ants and some bees and wasps. In their colonies, different physiological and behavioral functions are taken over by different groups of individuals: Queens monopolize reproduction and lay eggs, specialized nurses tend the brood, and foragers collect different resources and carry them to the nest (Wilson 1971). Foraging preferences of workers are regulated by their own as well as by colony-level demands (Csata & al. 2020). For instance, honeybee foragers preferably forage carbohydrate-rich nectar as long as most of the recently laid eggs are still capped in their cells and are not being fed (Traynor & al. 2015). When larvae begin hatching from these eggs and require larger amounts of protein, the proportion of foragers collecting protein-rich pollen increases although social insect foragers consume only small amounts of proteins themselves (Toth & Robinson 2005, Traynor & al. 2015).

Protein foraging in foragers serves as an excellent case study to understand the mechanisms of socially regulated foraging decisions because – in contrast to, for instance, species that provide parental care – foragers of most ant species do not reproduce. Hence, in most

species, foraging behavior is uncoupled and by that not confounded by changes in reproductive physiology. One exception are clonal ants such as *Ooceraea biroi*, in which all individuals of a colony, including foragers, can produce offspring (Tsuji & Yamauchi 1995, Ulrich & al. 2016). Brood-dependent protein foraging has not only been documented in bees but also in ants and wasps (Hoshikawa 1981, Cornelius & Grace 1997, Judd 2005, Dussutour & Simpson 2009), in which division of labor evolved independently (Peters & al. 2017). This repeated evolution suggests that socially regulated foraging preferences are important for maintaining colony homeostasis and are regulators of colony fitness.

In many social hymenopterans, foragers are not in direct contact with the brood to limit the spread of pathogens when returning from their foraging trips (Meunier 2015). Instead, brood carers take over food particles near the nest entrance and distribute them to the larvae (Buschinger & Schaefer 2006). This lack of direct larvae-forager interactions raises the question of how foragers adjust their foraging preference to meet larval demands. In honeybees, exposing the nest to e-beta ocimene, a pheromone released by young larvae (Maisonnasse & al. 2010), triggers an increase in foraging for protein-rich pollen (Traynor & al. 2015). That bees had no direct physical contact with the e-beta ocimene sample suggests that a passive diffusion of the volatile brood pheromone through the nest can trigger protein foraging in foragers. However, whether other social hymenopterans rely on a similar mechanism and whether larval cues include compounds such as e-beta ocimene that induce protein foraging is unknown.

In ants, the presence of brood has wide-ranging effects on worker behavior and physiology including the proportion of workers foraging (Ulrich & al. 2016), reproduction (Heinze & al. 1996, Ulrich & al. 2016), worker task choice (Starkey & Tamborindeguy 2023), and behavioral ontogeny (Starkey & Tamborindeguy 2023). These effects have partly been linked to larval chemical cues. For instance, exposing workers of the ant *Aphaenogaster senilis* to volatile larval chemical cues suppresses worker reproductive physiology and reduces the number of eggs laid (Villalta & al. 2015). Chemical profiles of ant brood are less complex than those of adult individuals (Schultner & Pulliainen 2020). In the leafcutter ant *Acromyrmex colombica*, worker profiles included hydrocarbons, aldehydes, acetates, alcohols, esters, and formats whereas larval profiles exclusively consisted of hydrocarbons (Richard & al. 2007). An ant brood pheromone has not yet been identified (see Schultner & Pulliainen 2020 for a detailed discussion). Despite this lack of complexity and larval specific pheromones, ant larval profiles can include information about viability (Dietemann & al. 2005), developmental stage (Richard & al. 2007), and caste (Penick & Liebig 2017) which potentially can impact worker foraging behavior. Besides chemical cues, worker foraging behavior can be influenced by larval behavior (Leonhardt & al. 2016, Schultner & Pulliainen 2020). Hungry larvae display a variety of behaviors including rocking the head back and forth and rhythmically opening and closing mandibula (Creemers & al. 2003, Buschinger & Schaefer 2006). These signals correlate with starvation (Peignier & al. 2019) and can induce worker feeding (Creemers & al. 2003, Buschinger & Schaefer 2006). Whether chemical and behavioral cues influence foraging preferences and if so, whether the simultaneous perception of both cues is necessary for changing foraging behavior in ants remains unknown.

To answer this, we used the acorn ant *Temnothorax longispinosus* as a model system and a combination of behavioral experiments, cuticular hydrocarbon (CHC) extractions and analyses, and quantifications of carbohydrate and protein foraging. We found that the presence of brood specifically increases protein but not carbohydrate foraging and that this effect could be induced solely by exposing a colony to larval CHC extracts, demonstrating that chemical cues are sufficient for increasing protein foraging.

Material and methods

Impact of brood on foraging preferences

Temnothorax longispinosus colonies were collected in April 2023 at the Edmund Niles Huyck Preserve in Rensselaerville, NY, USA. The species was identified using AntWeb (2024). At our laboratory at the University of Memphis, colonies were transferred to acrylic glass boxes measuring 14 cm × 14 cm × 4 cm containing a nest consisting of a plastic inlay covered with two glass slides and a moist paper tissue to increase humidity. Colonies were kept at a temperature fluctuation between +24 °C and +26 °C under a 12:12 light:dark photoregime and fed ad libitum with honey and crushed *Drosophila* flies to facilitate larval development. Brood of the colonies mainly consisted of 3rd and early 4th instar larvae (classified according to Buschinger & Schaefer 2006), which were observed being fed with fly particles, that is, solid protein (Fig. 1). It was first tested whether the presence of 3rd / 4th instar larvae induces changes in foraging preferences. Nineteen polygynous colonies with at least two queens, 54.0 ± 9.7 (mean ± standard error of the mean) workers, and 47.4 ± 11.8 3rd / 4th instar larvae were split into two similarly sized subcolonies. One of the subcolonies received all the brood, whereas the other subcolony was kept without brood. The subcolonies were starved for seven days to increase foraging activity. Following this starvation period, the subcolonies were offered two food sources at the same time for 60 min: honey as a carbohydrate source and crushed flies as a protein source. The number of foraging trips to each food source was counted. To control for variation in colony size, the per capita number of foraging trips to each food source was calculated by dividing the number of foraging trips to honey or flies, respectively, by the number of workers in the colony. It was then tested whether the presence of 3rd / 4th instar larvae influences carbohydrate and / or protein foraging. To this end, the per capita number of foraging trips in subcolonies without brood was subtracted from the per capita number of foraging trips observed

Fig. 1: Brood found in field-collected *Temnothorax longispinosus* colonies. (A) The colonies comprised of eggs (labelled as "E") and 3rd and 4th instar larvae (labelled as "L"). All larvae exhibited brown spots indicating that they were fed solid protein. (B) All larvae with brown spots had visible mandibula (triangle) required for consuming insect particles.

in subcolonies with brood. This was done separately for each food source, that is, once for carbohydrate and once for protein foraging. Then, a Generalized Linear Model (GLM) with a Gaussian distribution including the difference in per capita number of foraging trips as a response variable and the food source (carbohydrate, protein) as an explanatory factor was run. In addition, one-sided t-tests were used to test whether the difference in carbohydrate and protein foraging was significant. To test whether carbohydrate and protein foraging were correlated with each other, a Pearson's product-moment correlation test on the per capita carbohydrate and protein foraging across all subcolonies was run.

Transfer of larval CHCs

It was then assessed whether the observed impact of larval presence on protein foraging was mediated by volatile larval chemical cues diffusing through the nest. To this end, it was first confirmed that *Temnothorax longispinosus* foragers only rarely directly interact with brood and hence might rely on volatile chemical compounds released by the larvae, by re-analyzing data previously published by Kohlmeier & al. 2018. In that study, *T. longispinosus* colonies from the same population in Rensselaerville, NY, USA were fed with either RNA-interference (RNAi) against Vitellogenin-like A or DMSO only as a control, and the behavior and location of individually labeled young workers, brood carers, in-nest workers, guards, and foragers were documented on four consecutive days with five observations per day. Workers were classified as foragers if they were observed outside the nest prior to the nest scans, which has been found to be a precise method (Kohlmeier & al. 2017, 2018, 2019). Only colonies from the control treatment were used in this re-analysis to exclude RNAi dependent effects. Workers were grouped into foragers and non-foragers, and a Wilcoxon test was used to test whether the relative number of observations an individual spent on the brood was lower in foragers than in non-foragers. To then test the sufficiency of larval CHC extracts, 34 colonies with at least one queen, 34 ± 3.1 workers, and 44.7 ± 7.2 3rd / 4th instar larvae were chilled on ice, and all larvae were either collected in glass vials for CHC extractions (n = 17 colonies) or discarded as controls (n = 17 colonies). The number of larvae frozen ranged between 12 and 133. Glass vials were frozen at -20 °C, and colonies were starved for seven days. After the starvation period, larvae were defrosted for two minutes and CHCs were extracted in 200 µl n-hexane for 10 min. Extracts were pipetted onto a glass slide, and hexane was allowed to evaporate for five minutes. Glass slides were visually inspected to ensure that no residual hexane was left. The corresponding colonies from which the larvae were collected were chilled on ice for five minutes, and the top glass slide of the test colony nest was carefully removed and replaced with the glass slide covered with larval CHCs with the CHCs facing inwards. As a control, pure hexane instead of CHC extracts was used. Carbohydrate and protein foraging behavior was quantified as described above three hours after exposure. To test whether a transfer of CHCs influenced foraging behavior, one GLM with Gaussian distribution including the square-root transformed per capita number of foraging trips as a response variable, the food source (carbohydrates, protein), treatment (CHC, hexane), and their interaction as response variables was

used. Two Pearson product-moment correlation tests were used to test whether the number of larvae per extract influenced the per capita number of foraging trips to one of the two food sources.

Chemical analysis of cuticular hydrocarbon profiles

To compare cuticular extracts of adult workers and larvae, specimens from colonies were used that had been collected between July and September in 2021 in forests of four different US states (PA, NY, WV, OH) by E. Collin and M. Macit, such that each population was separated by at least 100 km. For each population, 12-18 colonies were collected within a 5 km radius. They were kept in artificial nest sites in a climate chamber at the University of Mainz (Germany), at 21 °C and a 12:12 light:dark cycle. They were fed *ad libitum* with water and an artificial diet similar to that used in the study of Bhatkar & Whitcomb (1970). For the CHC analysis, workers and larvae from different colonies but the same populations were used (n = 1-2 individuals per colony; total N = 35 and 33 for adults and larvae, respectively). They were placed individually into 1.5 ml glass vials and frozen at -20 °C. CHCs were extracted by immersing the individual in approximately 300 µl hexane for 10 min. The samples were then concentrated under a nitrogen flow down to approximately 20 µl. From each sample, 2 µl were injected into the gas chromatograph (7890A, Agilent Technologies, Santa Clara, CA) at 250 °C in splitless mode. Helium was used as carrier gas at a flow rate of 1.2 ml min^{-1}. The stationary phase was a Zebron Inferno ZB5-HT capillary column (30 m × 0.25 mm × 0.25 µm; Phenomenex Ltd, Aschaffenburg, Germany). The oven temperature started at 60 °C. After 2 min, it was increased by 60 °C min^{-1} up to 200 °C and then at 4 °C min^{-1} up to 320 °C, where it was held constant for 10 min. In the mass spectrometer (5975C, Agilent Technologies, Santa Clara, CA), the hydrocarbons were fragmented with an ionization voltage of 70 eV. The detector scanned for molecular fragments in a range of 40-550 atomic mass units. Data were acquired and processed with the software MSD ChemStation (E.02.02.1431, Agilent). All peaks larger than C20 were integrated and aligned manually. Hydrocarbons were identified according to retention index and diagnostic ions (Tab. S1, as digital supplementary material to this article, at the journal's web pages). Substances with a maximum (across all samples per species) below 0.5%, an average below 0.1%, and those that occurred in < 20% of the samples were excluded. The relative CHC composition was analyzed using PERMANOVA (command adonis2, 9999 permutations) with life stage and population as fixed factors. Relative abundance of *n*-alkanes was compared among life stages using linear mixed-effects models with population as random factor (n = 68 for all models). Since the *n*-alkanes were much more abundant in larvae, relative abundances of most other compounds were influenced by this *n*-alkane shift already. Therefore, we refrained from testing all other substances (and other substance groups) individually.

The DHARMa package was used to control for model fit and uniformity of residuals for all GLMs and Generalized Linear Mixed Models (GLMMs) (Hartig 2022): Table S2. All statistical analyses were performed in R v4.1.1 (R Core Team 2021) including the packages vegan (Oksanen & al. 2022), lme4 (Bates & al. 2015), and car (Fox & al. 2023), with $\alpha = 0.05$ as the cut-off value for statistical significance (Script S1).

Results

In split colonies, the difference in per capita number of foraging trips between subcolonies with and without brood was higher for protein than for carbohydrates (GLM: $\chi^2 = 8.0$; p = 0.004; Fig. 2A and Data S1). In the case of carbohydrate

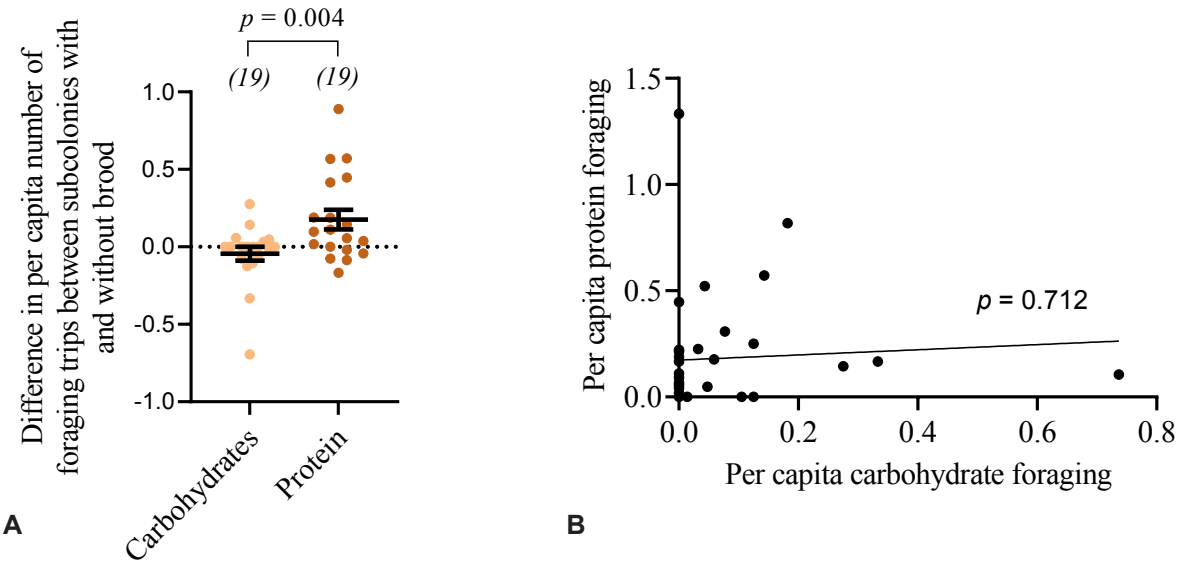

Fig. 2: Effect of brood presence on carbohydrate and protein foraging. (A) The presence of brood does not result in changes in the per capita carbohydrate foraging but increases per capita protein foraging. Bars display mean ± standard error. Numbers above each column display sample size. (B) No correlation between per capita carbohydrate and protein foraging was detected.

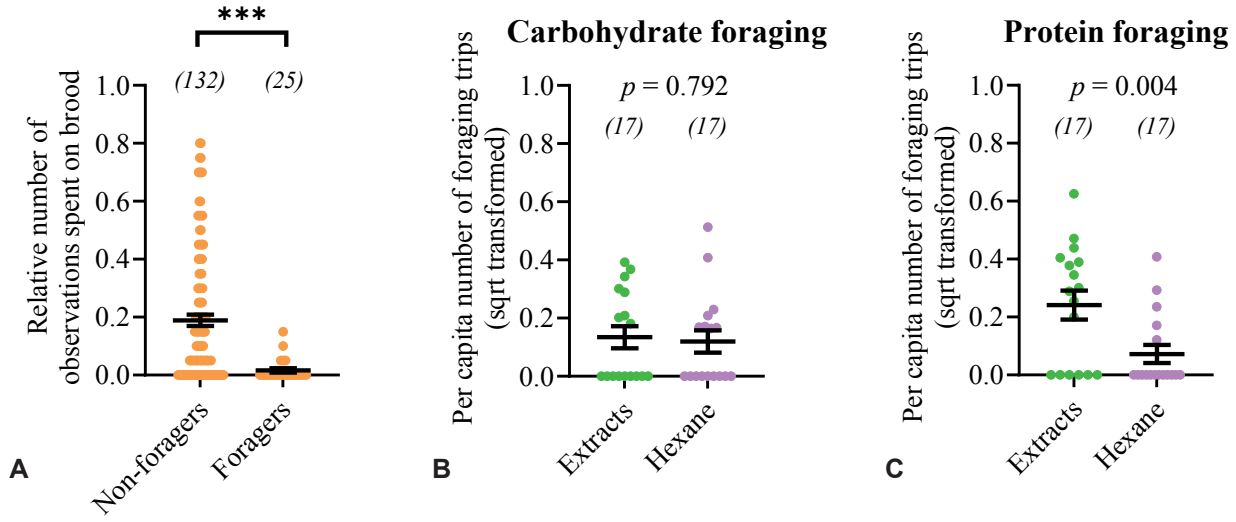

Fig. 3: Location of foragers and impact of larval chemical cues on foraging preferences. (A) Relative amount of time spent on brood. Foragers spent less time on brood than non-foragers. These data result from a re-analysis of a dataset in Kohlmeier & al. (2018). (B) The impact of larval CHC extracts (green) and pure hexane (pink) on carbohydrate and (C) protein foraging. Bars display mean ± standard error. Numbers above each column display sample size. Sqrt transformed = square root transformed.

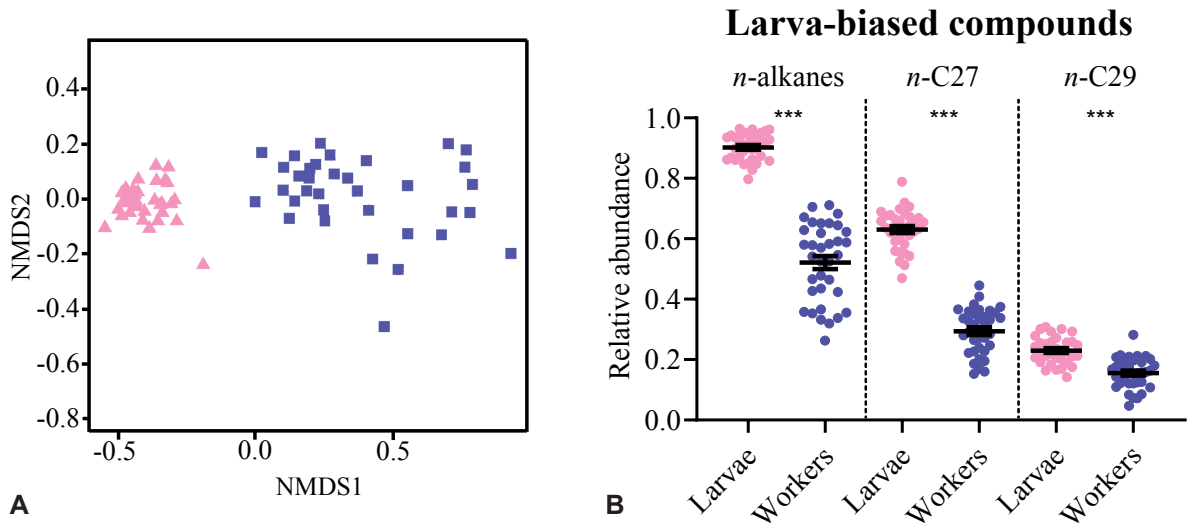

Fig. 4: GC-MS analysis of larval and worker CHCs. (A) NMDS plot of whole larval (pink triangles) and worker (purple squares) CHC profiles (STRESS = 0.0606). (B) Larval CHC profiles have higher relative abundances of *n*-alkanes and n-C27 than worker CHC profiles. Bars display mean ± standard error.

foraging, the difference in the per capita number of foraging trips was not significant (one-sided t-test: t = 1; p = 0.332), whereas in the case of protein foraging, this difference was significant (one-sided t-test: t = 2.8; p = 0.013). Per capita carbohydrate and protein foraging were not correlated with each other (Pearson: t = 0.4, degrees of freedom (d.f.) = 36, p = 0.712; Fig. 2B). We tested whether this lack of correlation was driven by the datapoint with the highest level of carbohydrate foraging by removing the datapoint and re-running the analysis. However, this did not change the result (Pearson: t = 1.0, d.f. = 35, p = 0.302).

Temnothorax longispinosus foragers spent only 1.6 ± 0.7% (mean ± standard error (S.E.)) of all observations on brood which is less than non-foragers, which spent 18.7 ± 0.02% of all observations on brood (Wilcoxon: W = 2614, p < 0.0001; Fig. 3A and Data S2). These data result from a re-analysis of data published by Kohlmeier & al. (2018) and confirm that foragers only sporadically interact with brood themselves. Exposing colonies to larval CHC extracts induced changes in foraging behavior 3 h after exposure (Fig. 3B, C and Data S3). We detected a trend for the per capita number of foraging trips being influenced by an interaction between food source and CHC exposure (GLM: χ^2 = 3.7, p = 0.053). An exposure to larval CHCs did not influence carbohydrate foraging (model summary: t = 0.3, p = 0.792), whereas

CHC exposure resulted in elevated protein foraging (model summary: t = 3.0, p = 0.004). The per capita foraging to carbohydrates and protein did not correlate with the number of larvae used for extractions (carbohydrates: Pearson: t = 0.6, d.f. = 15, p = 0.580; proteins: Pearson: t = 1.3, d.f. = 15, p = 0.213).

CHC profiles of larvae and workers comprised a total of 73 compounds including *n*-alkanes, mono-, di- and trimethyl alkanes; we did not detect any non-hydrocarbon compounds. We did not detect any larva- or worker-specific compounds that were present only in one life stage, neither CHCs nor non-hydrocarbon compounds (Fig. S1 and Data S4). However, the quantitative composition differed strongly between workers and larvae (PERMANOVA: $R^2 = 0.64$, $F_1 = 136.0$, p = 0.0001; NMDS: Fig. 4A) and also among populations ($R^2 = 0.06$, $F_3 = 4.3$, p = 0.0022). Most notable was that larval profiles contained a much higher proportion of *n*-alkanes than worker profiles (90.2 ± 0.8% versus 52.2 ± 2.2%; GLMM: $\chi^2_1 = 318.8$, p < 0.0001; Fig. 4B). This was due to *n*-C27, which represented 63.0 ± 1.1% of all compounds in larvae, compared with 29.4 ± 1.3% in workers (LMM: $\chi^2_1 = 469.32$, p < 0.0001; Fig. 4B), and *n*-C29, which was more abundant in larvae as well (larvae: 30.0 ± 0.01%; workers: 15.5 ± 1.7%; $\chi^2_1 = 41.4$, p < 0.0001). In addition, *n*-C26 was more abundant in larvae than in workers (larvae: 1.21 ± 0.2%; workers: 0.85 ± 0.08%; Wilcoxon test: W = 353, p = 0.0055). All other *n*-alkanes had abundances below 3% in either stage; among these, *n*-C28 and *n*-C31 were more abundant in workers than in larvae (both $\chi^2_1 > 19$, p < 0.0001).

Discussion

In many social systems in which cooperative behaviors evolved, only a subset of all individuals forages. These foragers collect nutrients and transport them to the nest not only to meet their own nutritional needs but also to satisfy the demands of their nestmates. It can thus be predicted that individuals that remain in the nest, for example, immobile offspring, emit cues associated with their nutritional demands to their foraging nestmates. We used *Temnothorax longispinosus* ants to test whether the presence of 3rd / 4th instar larvae, which are fully dependent on being fed by adult nestmates, increases protein foraging and to explore whether the perception of larval volatile chemical cues is sufficient to induce changes in the foraging preferences of workers. Our findings show that colonies with larvae exhibit greater protein foraging compared with colonies without larvae, while carbohydrate foraging is not influenced by brood presence. Exposing brood-less colonies to CHC extracts of larvae was sufficient to stimulate an increase in protein foraging. Chemical profiles of larvae and workers did not include any detectable lifestage-specific compound, but larval profiles exhibited higher proportions of *n*-alkanes, in particular of *n*-C27 and *n*-C29.

Our findings align with previous studies conducted on social hymenopterans, which have demonstrated that foraging decisions are influenced by various factors, such as predation risk (Barbee & Pinter-Wollman 2022), seasonality (Cook & al. 2011), and starvation (Csata & al. 2020). Brood-dependent changes in foraging activity and foraging preferences have been described in multiple species, including honeybees (Traynor & al. 2015), bumble bees (Kraus & al. 2019), wasps (Ishay & Landau 1972), and ants (Dussutour & Simpson 2009, Ulrich & al. 2016). To our knowledge, our study provides the first demonstration that chemical cues of larvae are sufficient to rapidly, that is, within 3 h, induce protein foraging in ants. Larvae can impact reproduction (Heinze & al. 1996, Schultner & al. 2017, Chandra & al. 2018), worker gene expression (Warner & al. 2019), brood care behavior (Snir & al. 2022), the defense against social parasites (Pulliainen & al. 2019), and the digestion of food through the salivary secretions (Schultner 2019). Our novel findings that larval chemical cues influence foraging preferences hence add up to increasing evidence that larvae take over a pivotal role in regulating social organization and behavior in ants.

Larval CHC extracts are sufficient to increase protein foraging. Our GC-MS analysis of larval and worker CHC extracts did not reveal any larvae-specific compound but suggests that ant workers rely on quantitative rather than qualitative differences for the previously documented ability to distinguish between larvae and workers based on chemical cues alone (Kohlmeier & al. 2018). However, we cannot rule out the possibility that larvae emit a signal that was not detected by our GC-MS analysis. The high proportion of *n*-alkanes in larval profiles is consistent with what has been found in other ant species (Villalta & al. 2016, Schultner & al. 2023). For instance, in *Tetramorium semilaeve*, 92.5% of all compounds found in larval profiles are either *n*-C25 or *n*-C27 (Salazar & al. 2015). One reason for this high proportion of *n*-alkanes in larval profiles might be linked to desiccation resistance: The cuticle of larvae is less sclerotized than that of workers and may thus be more permeable for water. High proportions of *n*-alkanes might hence improve waterproofing but might at the same time serve as a signal for workers to differentiate larvae and adults. Larval CHC profiles can differ even between closely related ant species and are often qualitatively similar to those of the respective adult workers (Elmes & al. 2002). This is also supported by this study and indicates that different species might rely on different compounds (or combinations thereof) to recognize larvae and adjust their protein foraging. Alternatively, the high abundance of *n*-alkanes might serve as a larval signal across species, but this needs further confirmation. Whether all components of the larval CHC profile are volatile or whether only a subset of compounds diffuses through the nest should be addressed in the future, for example, by analyzing the chemical composition of the larval headspace. Even long-chain hydrocarbons up to C29 are volatile to some extent and can be found in the gas phase of insects (Schmitt et al. 2007). This reliance on passive diffusion of larval cues through the nest might be attributed to the relatively small colony and nest size of *Temnothorax longispinosus*, which typically nests in acorns or short galleries in sticks. It would be interesting to

see whether ant species that inhabit larger nest sites, where the physical distance between brood and foragers exceeds a few millimeters to centimeters, have evolved systems that actively convey cues such as cuticular hydrocarbons from larvae to foragers, for example, via trophallaxis between brood carers and foragers (LeBoeuf & al. 2016, Meurville & LeBoeuf 2021). However, we cannot fully rule out the possibility that such an active transport of cues via brood carers does influence protein foraging as brood carers might have licked off larval extracts from the glass slide and conveyed them to the foragers via trophallaxis, although we did not observe such behaviors during our experiments. That CHC extracts alone can trigger protein foraging raises the question of the function of additional forms of begging, for instance, via acoustic or visual cues (Leonhardt & al. 2016, Schultner & Pulliainen 2020). In *Leptothorax acervorum*, an ant species closely related to *T. longispinosus*, larvae respond to antennation by rocking back and forth and by opening and closing their mandibula (Buschinger & Schaefer 2006). We observed mandibula movements in *T. longispinosus* larvae when fly particles were brought to the nest and their distribution to larvae began (P. Kohlmeier, unpubl.). One possible scenario is that larval chemical cues induce protein foraging whereas begging for food, for example, via locomotive or acoustic cues, motivates and guides brood carers to deliver food particles to the respective larva. More research will be required to dissect the effects of larval chemical, behavioral, and other, such as acoustic, cues on worker behavior. We observed that the exposure of a colony to larval CHC extracts increased protein foraging (Fig. 3) but not to the same level as when a colony contains actual live larvae (Fig. 2). One explanation might be that chemical and visual / acoustic begging cues have additive effects on protein foraging. Alternatively, the concentration of volatile compounds diffusing through the nest might be lower when using extracts compared with live larvae, chemical compounds might require more time than the three hours in our experiments to fully activate protein foraging or are not stable over the full duration of the experiment. Some of the colonies exposed to larval CHC extracts did not show any foraging activity (Fig. 3C). Such lack of foraging activity even after a starvation period has been observed before in *T. longispinosus* (Kohlmeier & al. 2018) and might represent a lab artifact as colonies might have been foraging extensively when being fed *ad libitum* prior to the onset of the observations and do not require new resources immediately.

We found no evidence of a positive or negative correlation between per capita carbohydrate and protein foraging. This suggests that, at the colony level, protein foraging can be increased without altering carbohydrate foraging and that there is no trade-off between these two types of foraging. This allows a colony to independently regulate the intake of different nutrients and dynamically adapt to changes in nutritional demands, thereby maintaining homeostasis (Dussutour & Simpson 2009). It remains unclear whether the observed increase in protein foraging is a result of recruiting additional protein-foragers or of individual changes in the foraging preferences of already active foragers. It is possible that newly recruited foragers specialize in protein foraging while not contributing to carbohydrate foraging or that existing foragers increase their investment in protein foraging without altering their investment in carbohydrate foraging. In insects with a less complex social behavior like fruit flies, a shift from carbohydrate to protein foraging is induced by mating (Ribeiro & Dickson 2010). Thus, one hypothesis is that in social insects, these two behavioral programs have become linked to express both of them in unmated workers and are no longer regulated by mating status but by the social environment.

In conclusion, our findings demonstrate that colonies can rapidly respond to changes in nutritional demands and that chemical cues might be the primarily used means to communicate these changes. The ability to quickly adjust foraging behavior contributes to colony homeostasis and hence colony fitness, and this further highlights the important role of larvae in regulating colony and individual worker behavior.

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