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Odor-Mediated Group Organization and Coordination in the Termite-Raiding Ant *Megaponera analis* (Mayr)

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Abstract

Visual and olfactory communications are vital for coordinated group hunting in most animals. To hunt for prey, the group-raiding termite specialist ant *Megaponera analis*, which lacks good vision, must first confirm the presence or absence of conspecific raiders. Here, we show that *M. analis* uses olfactory cues for intraspecific communication and showed greater preference for conspecific odors over clean air (blank) or odors from its termite prey. Chemical analysis of ant volatiles identified predominantly short-chained hydrocarbons. Electrophysiological analysis revealed differential sensory detection of the odor compounds, which were confirmed in behavioral olfactometric choice assays with odor bouquets collected from major and minor castes and the 2 most dominant volatiles and *n*-undecane *n*-tridecane. A comparative analysis of the cuticular hydrocarbon profile with those of the short-chained odor bouquet of different populations shows a high divergence in the long-chained profile and a much-conserved short-chained odor bouquet. This suggests that there is less selection pressure for divergence and individual recognition in the short- than the long-chained odor profiles. We conclude that olfactory communication serves as an alternative to visual or sound communication, especially during group raids in *M. analis* when ants are not in direct contact with one another.

Key words: cuticular hydrocarbons, foraging, group hunt, olfactory communication, *Pachycondyla analis*

Introduction

Cooperative hunting allows predatory species to find and subdue larger or more numerous prey with greater efficiency than when foraging alone. It is believed to play a central role in the evolution of sociality and advanced cognitive abilities (Bailey et al. 2013) and necessitates a high degree of communication (Lang and Farine 2017). In most taxa, this information transfer is conveyed visually or vocally to indicate position, presence/absence of group members,

and the onset of the hunt. These modes of communication during a hunt have been studied in a wide range of taxa, such as Lionfish (Lonnstedt et al. 2014), Carnivora (Bailey et al. 2013), or chimpanzees (Boesch 1994). In the absence of these senses, different modes of communication have evolved to improve group hunting, like eavesdropping in echolocating bats (Dechmann et al. 2009).

In ants, visual cues are rarely used for intraspecific communication but are mainly used for orientation and landmark navigation (Wehner et al. 2014). Vocal cues are also limited to stridulatory sounds mostly used as a distress signal. Where vision and sounds provide too limited information, chemical communication forms the major means of communication in eusocial insects and plays a central role in the organization of their societies (Hölldobler and Wilson 1990). These roles include bringing individuals temporarily together at relevant locations and recruiting colony members for the efficient utilization of available resources. Collective exploitation and aggressive and/or defensive behaviors are also controlled by chemical signals with such behavioral traits presumed to be crucial in ecological dominance in invasive ant species (Holway et al. 2002). Recognizing and discriminating between group and nongroup members constitutes a very important ecological trait in ant societies as nongroup members pose a serious threat to colony resources and ultimately its survival (Guerrieri et al. 2009; Yusuf et al. 2010).

Social insects are extremely effective at coordinating their movements in time and space to optimize foraging patterns in stochastic environments. In most cases, these food sources necessitate only a limited amount of coordinated cooperation for exploitation in order to forage optimally, especially in solitary hunting species. However, this is not so for specialist group-hunting species where there is the need to make quick, coordinated decisions. One such specialist is the termite-raiding ant *Megaponera analis*.

Megaponera analis (Mayr) is a specialized termite predator widely distributed in sub-Saharan Africa (Levieux 1966; Schmidt and Shattuck 2014; Frank and Linsenmair 2017a). This ant species, commonly referred to as "Matabele ants," organizes highly coordinated group raids on termite species that belong to the subfamily Macrotermitinae (Longhurst et al. 1978; Frank and Linsenmair 2017a). After a scout has found a suitable food source, it will recruit 200–800 individuals to a site where termites are foraging. These ants will follow the scout in a distinct column formation with a van and rear guard (Frank and Linsenmair 2017b). Shortly before arrival, the raiders will wait for the column to gather and attack together. The hunting phase itself is divided into 3: a first phase of breaking through the protective soil cover over the termite galleries; a second phase in which the smaller ants rush into the openings to hunt and carry out the prey; and a third phase when the immobilized termites are gathered by the larger ants. The different phases rarely last longer than 4–5 min after which the ants gather again at the starting point of the raid, preparing to return together in the same column formation to the nest (Longhurst et al. 1978; Lepage et al. 1981; Bayliss and Fielding 2002; Yusuf, Gordon, et al. 2014; Frank and Linsenmair 2017b).

Chemical communication within and outside the ant's nest, particularly during raids on termites has not been well described in *M. analis*. Previous studies reported the use of trail-laying signals possibly released from glandular sources for communication (Longhurst et al. 1978; Holldobler et al. 1994; Janssen et al. 1995). Recently, we showed that cuticular hydrocarbons (CHCs) mediated nestmate recognition in this ant species (Yusuf et al. 2010). In addition, injured ants attract helpers after the hunt to their location through pheromones in the mandibular gland (Frank et al. 2017). However, the well-coordinated raiding behavior of *M. analis* on termite-foraging sites suggests the involvement of a complex chemical communication system for both nestmate and caste recognition and, to some extent, task allocations. This coupled with the observations that different worker castes played different roles during raids prompted us to explore the possible involvement of olfactory cues in raid coordination of *M. analis* workers. Here, we show that airborne volatile odors, mainly short-chained hydrocarbons, continuously emitted by the ants, are involved in communication between raiding ants and is vital for the coordination of the raid.

Materials and methods

Insects

Three colonies of *M. analis,* identified by Marcus Stüben (University of Würzburg, Germany), with representatives of both sexes (workers and males), eggs, cocoons, and larva, were excavated at Mpala (0°17'N, 37°52'E) on the research facility of Mpala Wild Life Foundation Central Kenya. These were transported to the Animal Rearing and Containment Unit located on the *icipe* Duduville campus in Nairobi (1°22'S, 36°89′E), Kenya. The ant colonies were placed in nesting boxes (20 × 20 × 20 cm) made of aluminum with a removable lid to observe the colony (Yusuf et al. 2013). The base of the nesting box was partially filled with soil from the excavated ant's nest that served as nesting material. This was attached to a foraging arena (1.0 × 1.5 m) made of Perspex that was also partially filled with sterilized soil. This soil was thoroughly washed with double-distilled water and oven-dried overnight at 160 °C.

Ants were fed with termites (mainly of the subfamily Macrotermitinae) collected from mounds or foraging galleries around the *icipe* Duduville campus. Feeding was carried out twice daily in the morning and evening. Conditions in the rearing room were kept between 50% and 60% relative humidity (RH) and 24–29 °C under a natural photoperiodic cycle of 12:12 h light:dark.

For comparative chemical analyses, ants were also collected from a population in the Comoé National Park, Côte d'Ivoire, in the vicinity of the Comoé National Park Research Station (8°46'N, 3°47'W) and from a population in Gorongosa National Park (18°58'S, 34°21'E), Mozambique in the vicinity of the E.O. Wilson Laboratory.

Classification of workers

Since workers of *M. analis* exhibit monophasic allometry (Crewe et al. 1984; Villet 1990), workers were classified into 2 groups (major and minor) using the following morphological characters: interocular distance, body, and scape length as described (Crewe et al. 1984). Workers falling into classes 1–5 were grouped as minor and those in classes 6–10 as major workers.

Volatile collections

To determine the olfactory cues used for intercaste communication by *M. analis* workers, volatiles were collected separately from major and minor workers and a combination of the 2 castes in the following treatments: 1) 20 majors; 2) 10 majors and 10 minors (mixed workers); and 3) 20 minors. The ants were placed in 2-L cylindrical glass containers with single-port lids (Analytical Research Systems INC) and charcoal-purified humidified air was passed over the ants through a Super Q trap (30 mg) at 0.5 L/min for 22 h as described in Yusuf, Crewe, et al. (2014). Prior to connecting the adsorbent traps, the collection chambers were purged

by passing humidified air through them for 20 min to allow the ants to settle down in the containers and to flush out potential alarm pheromones released during the handling of the ants. Each filter was eluted with 200 µL of GC-grade dichloromethane (Sigma Aldrich) and the eluent stored at –20 °C prior to analysis. This procedure was repeated using worker ants from 3 different colonies.

The CHC profile of workers from Côte d'Ivoire and Mozambique were extracted for 10 min in 1 mL of Hexane. Afterward, the extracts were transported to the University of Würzburg (Germany) and stored at –20 °C until use.

Analyses of odors

To determine the qualitative and quantitative composition of odors and CHCs from different caste and populations, volatile and CHC extracts were analyzed by coupled gas chromatography–mass spectrometry as follows.

Ant volatiles

Coupled gas chromatography–mass spectrometric analysis of the Super Q volatile extracts of workers were carried out on an Agilent Technologies 7890A GC equipped with an HP-5 MS capillary column (30 × 0.25 mm ID × 0.25 µm film thickness) coupled to a 5795C MS. One microliter of each sample was injected into the GC in a splitless mode, with helium used as the carrier gas at a flow rate of 1.0 mL/min. The oven temperature was 35 °C for 5 min, increased to 280 °C at 10 °C/min, and then held at this temperature for 15 min. Spectra were recorded at 70 eV in the electron impact (EI) ionization mode. Compounds in the ant volatiles were identified by comparing their mass spectral data with those in the library (NIST11). The identities of the *n*-alkanes were confirmed by coinjection and comparison of MS data with those of authentic standards. Compounds present in the volatiles were quantified using 1-heptadecene as an internal standard.

Cuticular hydrocarbons

CHC extracts were evaporated to a volume of approximately 100 µL and 1 µL was analyzed by using a 6890 gas chromatograph coupled to a 5975 mass selective detector Agilent Technologies. The GC was equipped with a DB-5 capillary column (0.25 mm ID × 30 m; film thickness 0.25 µm, J & W Scientific). Helium was used as a carrier gas at a flow rate of 1 mL/min. A temperature program from 60 to 300 °C with 5 °C/min and finally 10 min at 300 °C was employed. The mass spectra were recorded in the EI mode with an ionization voltage of 70 eV and a source temperature of 230 °C.

The software ChemStation version (Agilent Technologies) for windows was used for data acquisition. Identification of the components was accomplished by comparison of library data (NIST 11) with mass spectral data of commercially purchased standards and diagnostic ions.

Chemicals

n-Nonane, *n*-decane, *n*-undecane, and *n*-dodecane *with* a purity of >99% were obtained from Sigma Aldrich Chemical Company. *n*-Tridecane, *n*-tetradecane, *n*-pentadecane, and *n*-hexadecane *were provided by the late Dr. Peter Teal, USDA/ARS-Centre for Medical, Agricultural and Veterinary Entomology*.

Olfactometer

The olfactometer consisted of a glass Y-tube (base, 7.5 cm long; Y-arms, each 7.5 cm long; internal tube, 10 mm outer diameter). The Y-tube apparatus was modified after the design of (Carroll et al. 2006) and bioassays were conducted as previously reported in Yusuf, Crewe, et al. (2014) with slight modifications. Briefly, the 2 arms and base tube of the olfactometer were connected to Teflon tubes similar in size and were, in turn, attached directly to the odor source and vacuum pump. A mesh screen was placed at each end of the olfactometer to prevent ants from entering the Teflon tubes. Odor sources were placed in 200-mL glass chambers with screw tops containing inlets for incoming air and outlets for odors to exit into the Y-tube. Charcoal-purified air was passed into the odor chambers at a flow rate of 250 mL/min. One of the Y-arms was connected to an odor source, while the other was connected to an empty jar with only clean air (blank) passing through it. The odors were extracted through the base arm at 500 mL/min by a vacuum pump to ensure a constant flow and to prevent odors from building up in the Y-tube.

Bioassays with living ants

To answer the question if olfactory cues are used by ants for communication, we tested the responses of major and minor workers of *M. analis* to conspecific odors in a Y-tube olfactometer. The odor sources consisted of 1) 20 major, 2) 20 minor, and 3) a combination of 10 major and 10 minor workers against clean air used as control. To rule out possible bias in relation to the limited choices of odors presented, we tested the response of workers to their own odors against those of termites in their galleries (positive control), an odor source to which the ants respond to (Yusuf, Crewe, et al. 2014). The bioassays were conducted at room temperature (24 ± 1 °C) and 50–60% RH. In order to conform to the circadian rhythms of foraging and raiding behavior, all bioassays were carried out in the mornings and evenings (during the period 7 AM to 10 AM and 4 PM to 5.30 PM local time, respectively) using ants from 3 different colonies. Prior to bioassays, the odor source chambers with ants were purged by passing air through them for 10 min to allow the ants to settle down in the containers and to flush out potential alarm pheromones released during the handling of the ants.

Test ants were introduced individually by disconnecting the Y-tube at its base and allowing the ant to walk into the olfactometer. Subsequently, the tube was reconnected to reestablish airflow from the odor sources through the arms and out at the base toward the vacuum pump. The ant was allowed to settle down for 5 min, after which its behavior was monitored. A choice was recorded when an ant stayed for at least 1 min in an arm or when it frequently visited an arm. A no-choice response was recorded when the ant remained in the base arm for more than 5 min. Each test was terminated 10 min after the introduction of the ant into the Y-tube. Sixty replicates were carried out for each treatment (30 minor and 30 major workers). To avoid positional bias, odor chambers were switched for every replicate. A clean Y-tube was used for each replicate in order to avoid contamination from trail pheromones. All the Y-tubes were thoroughly cleaned with a scent-free detergent and water, rinsed with acetone, and dried in the oven for 5 h at 160 °C. The Teflon tubes were washed with water and detergent, rinsed with double-distilled water, and then dried in a stream of nitrogen.

Bioassays with volatile extracts

Next, we tested whether ant responses to volatiles were dose dependent. The number of ants used for this collection was based on the results obtained from the analysis of volatiles collected from living ants. Volatiles were collected from 50 worker ants, comprised of 25 majors and 25 minors for 8 h using procedures described earlier with living ants. The adsorbent trap was eluted with 400 µL of dichloromethane. Five concentrations, including 12.5, 25, 50, and

100 ant hours (an ant hour is equivalent to the amount of volatiles released by a major or minor ant in an hour), were prepared from the stock solution. Each dose was, loaded on to a rubber septum, air-dried, and then transferred into a 200-mL glass container and tested individually as an odor source against clean air (control). Forty ants (20 majors and 20 minors) were used for each concentration making a total of 160 ants from 3 colonies for the assays.

Bioassays with *n*-undecane *and n*-tridecane

Previously *n*-undecane and *n*-tridecane were shown to elicit alarm responses in workers of M. analis in the field (Longhurst et al. 1979). To demonstrate the roles of these compounds in olfactory communication in worker castes, we tested responses of major and minor ants to these 2 major components identified in their volatiles at varying concentrations: 0.125, 0.25, 0.5, and 1.0 ng/septa in the olfactometer as previously described. Preparation of the different concentrations of each compound was done similarly as described for the extracts. For each concentration of synthetic compound tested, responses of 40 ants (20 majors and 20 minors) were recorded and 320 ants from 3 different colonies used for these assays.

Electroantennography

To determine if volatiles from workers are detected by ant antennae, electroantennographic analyses were performed with the extracts and the elicited responses recorded. A silver wire inserted into the ant's head capsule served as reference electrode. A glass capillary filled with potassium chloride solution and connected to a silver wire was positioned at the tip of the antennae, which was previously cut off. The electrode signal was 10× preamplified at the head stage (Neuroprobe Amplifier 1600, A-M Systems), high-pass filtered (Kemo VBF 8, Kemo Inc.), and digitalized by an acquisition board (Labtrax 4/16, WPI). Data were recorded with LabScribe 3 (WPI) at a sampling rate of 1 kHz.

The compounds were applied using a stimulus controller (Stimulus Controller CS-55, Syntech) generating a continuous airflow of 1 L/min added with a stimulus flow of 0.5 L/min. Two stimulus chambers were inserted into the air stream (stimulus chamber 1 and 2). Prior to odor stimulation, an airflow of 0.5 L/min was blown over an untreated filter paper placed in stimulus chamber 1. For providing the stimulus, the airflow switched from stimulus chamber 1 to stimulus chamber 2, equipped with a filter paper treated with the test compound. After 0.5 s of stimulation, the airflow was switched back to stimulus chamber 1. All stimuli were presented 3 times per individual in a pseudorandomized order.

Statistical analyses

To visualize the data for chemicals from the 3 populations of *M. analis*, a nonmetric multidimensional scaling was used. A permutational multivariate analysis of variance using distance matrices using the ADONIS function in R was performed to test for differences between populations and colonies of *M. analis*. To test if ants exhibited sensory responses to C10–C17 hydrocarbons, data for the electroantennography (EAG) were subjected to a Wilcoxon Rank Sum test and the contributions of these compounds to the EAG visualized using a principal component analysis. Data for the different bioassays were analyzed using a 1-sample χ^2 test where the number of ants responding to the test odor source was compared to those responding to the control (clean air) or termite gallery odor where appropriate. Nonresponding ants were excluded from all analysis to preclude bias as they do not contribute to the test. All statistical analyses were carried out using R 2.12.0 (R Development Core Team 2010).

Results

Analysis of volatiles

Volatiles were released by all worker castes of *M. analis*. Analysis of these volatiles revealed that major, minor, and mixed (minors and majors) worker groups produced volatiles that were identical in composition. These comprised mainly of straight- and branched-chain saturated hydrocarbons, with chain lengths between C_9 (nonane) and C_{17} (heptadecane; Figure 1; Supplementary Figure S1). However, the proportions of these compounds varied between castes and population (Figure 1). The volatile production pattern was similar for the colonies from Kenya and the 2 populations of *M. analis* from Mozambique and Côte d'Ivoire. The profiles showed similar qualitative composition of straight and branched saturated hydrocarbons, with *n*-undecane (C11) and *n*-tridecane (C13) being the major components (Figure 1; Supplementary Table S1).

Differences in cuticular and odor profiles between colonies and populations

To determine whether the CHCs of the different ant populations were conserved, volatile and CHC profiles of *M. analis* from Kenya, Côte d'Ivoire, and Mozambique were analyzed. The chemical profiles identified individual groups and their colonies of origin for the ant populations from Côte d'Ivoire and Mozambican (Figure 2; ADONIS: Côte d'Ivoire: $F_{4,20} = 33$, $R^2 = 0.89$, $P < 0.001$; Mozambique: $F_{4,24} = 5.68$, $R^2 = 0.53$, $P < 0.001$). The CHC profiles showed qualitative and quantitative differences (Figure 2; ADONIS: population: $F_{2,50} = 134.8$, $R^2 = 0.85$, $P < 0.001$). These differences were less apparent in the odor plume of *M. analis* from Kenya, explaining only 25% of the variance compared to 85%

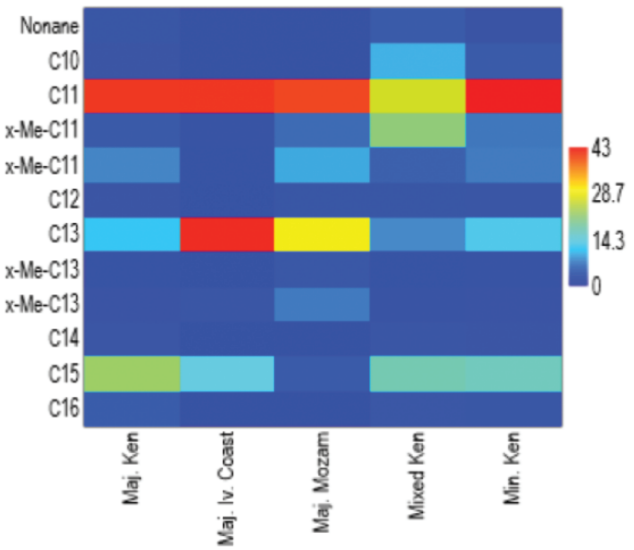

Figure 1. Composition of individual components from volatiles and CHCs from different populations of *Megaponera analis*. Maj. Ken (major workers from Kenya), Maj. Iv. Coast (major workers from Cote d' Ivoire), Maj. Mozan (major workers from Mozambique), Mixed Ken (mixed workers from Kenya), and Min. Ken (minor workers from Kenya). Individual components are shown on *y* axis; C10-C17 are decane, undecane, dodecane, tridecane, tetradecane, pentadecane, hexadecane, and heptadecane, while x-Me-C11 and x-Me-C13 are methyl branched undecane and methyl tridecane, respectively

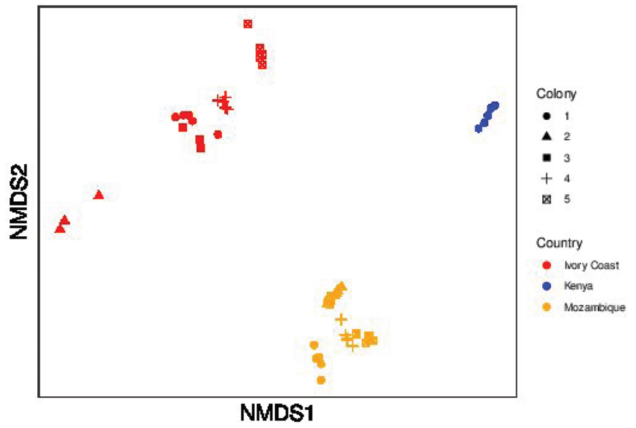

Figure 2. A nonmetric multidimensional scaling plot of the chemical profiles comparing populations and colonies from Côte d'Ivoire (N = 21), Mozambique (N = 25), and Kenya (N = 5).

in the CHC profile (Figure 2; ADONIS: population: $F_{1,34}$ = 11.3, R^2 = 0.25, P < 0.001). Whereas minor differences were detected between the odor plumes of colonies in the Côte d'Ivoire populations (Figure 2; ADONIS: colony: $F_{2,10}$ = 3.96, R^2 = 0.49, P > 0.05), no significant differences were detected in the odor plumes of the Mozambican populations (Figure 2; ADONIS: colony: $F_{4,23}$ = 0.94, R^2 = 0.16, P > 0.05).

Sensory response to short-chained hydrocarbons

Electrophysiological assays showed that *n*-decane *(C10)*, *n*-undecane *(C11)*, *n*-tridecane *(C13)*, *n*-pentadecane *(C15)*, and *n*-heptadecane *(C17)* elicited significant (signed-rank test; P < 0.001) antennal responses (Figure 3A–D). A plot of the first 3 principal components of the recorded EAG responses indicated that the different hydrocarbons induced distinct receptor activity and, together, the C10–C17 accounted for 88% of these differences (Figure 2E).

Behavioral response to ant volatiles

To answer the question if ants use olfactory cues from conspecifics and different castes for intraspecific and interspecific communication during raids or in their nest, we tested the responses of ants to odors from different castes. Both major and minor workers responded to odors of their conspecifics (Figure 4). Overall, more than 70% of the ants chose the odors from the mixed conspecifics (Figure 4A). The responses of major and minor workers to odors of the mixed workers were significantly higher than the responses to control (clean air; χ^2 = 9.8, degrees of freedom [df] = 1, N = 30, P < 0.01, majors; χ^2 = 14.4, df = 1, N = 30, P < 0.001, minors). Similarly, the responses of major workers to their own odors (χ^2 = 6.8, N = 30, P < 0.01) and minor workers to their own volatiles (χ^2 = 14.29, df = 1, N = 30, P < 0.001) were significantly higher than responses of the ant castes to the control (clean air; Figure 4B,C). When workers were presented with a choice between their own odors and those from their termite prey (Figure 4D), they showed a preference for conspecific odors, especially for major workers (χ^2 = 3.80, df = 1, N = 60, P < 0.01). Because responses of both major and minor workers to odors from conspecifics were similar to those to the control (clean air), the data for all workers from subsequent assays were pooled together.

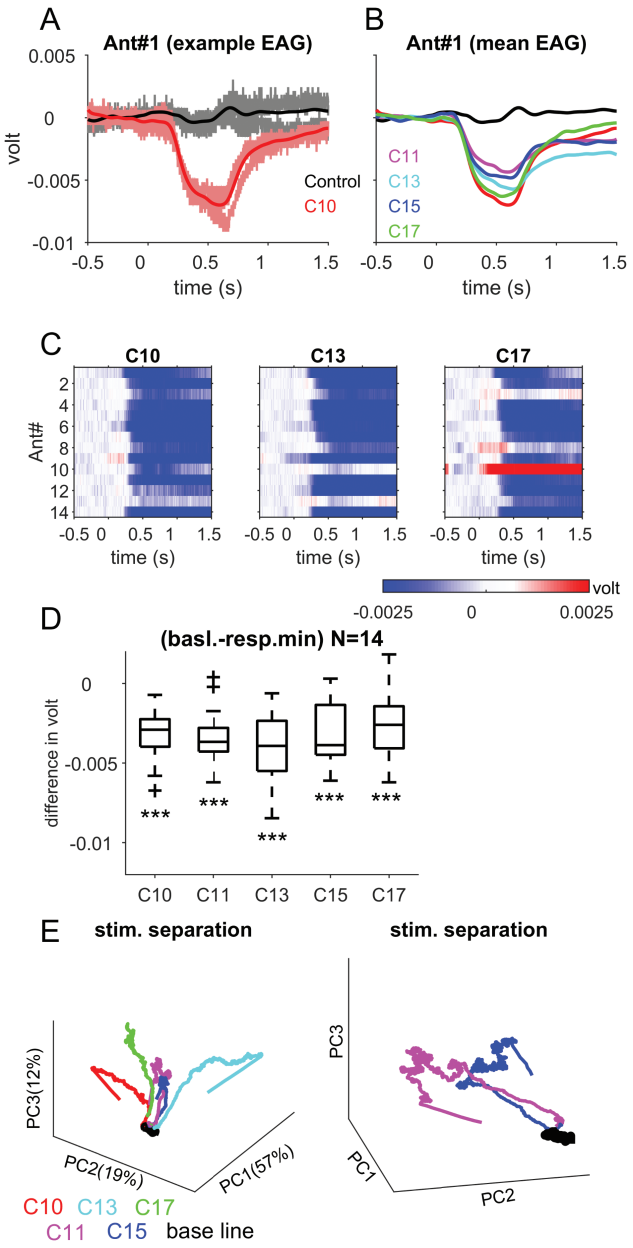

Figure 3. EAG recordings of ants' responses to C10-C17 odors. All tested straight-chained hydrocarbons elicited significant antennal responses. **(A)** The EAG signals of 3 C10 repetitions (pink) and their average (red) is shown. The 3 replicates of the control (gray) and their average (black) remained at the baseline. Stimulation started at time 0 and lasted for 500 ms. **(B)** The 3 repetitions of each odor were averaged in each insect (the shown example is the same as in (A)). **(C)** Overview of the 14 insects tested. Each line corresponds to the color-coded mean activity of the 3 replicates per stimulus. Stimulation induced typically negative EAG signals. **(D)** In each insect, we calculated the difference between the minimal baseline activity in the 500 ms before odor onset and the stimulus induced minimal EAG in the first 1000 ms of stimulation. The distribution for all stimuli was significantly different from 0 (singed rank test; P < 0.001). **(E)** The population vectors (as shown in (C)) of all stimuli were used in a principal component analysis. Plotting the first 3 principal components revealed distinct EAG activity for all tested molecules. The variation explained by the first principal components is given in the axes. An enlargement of and slight turn of the 3D plot is shown to illustrate the separation between C11 and C15 (right).

Behavioral responses of workers to different doses of volatile odors

To determine whether responses of workers to volatiles were adaptive, we tested the responses of workers to different concentrations of ant volatiles in Y-tube assays. All workers responded to the concentrations of ant volatiles preferring these over the control (clean air; Figure 5; $\chi^2 = 23.00$, df = 1, $N = 160$, $P < 0.001$).

Behavioral response to *n*-undecane *and n*-tridecane

To demonstrate the roles of chemical components from the odor profiles of *M. analis*, we selected the 2 main components *n*-undecane and *n*-tridecane that were shown to be part of the ants alarm pheromones for dose-response tests against workers. Workers responded differently to *n*-undecane *and n*-tridecane (Figure 6), preferring *n*-undecane to the control at all concentrations except at 1.0 ng (Figure 6A; $\chi^2 = 5.05$, df = 1, $N = 30$, $P < 0.05$). However, they only preferred *n*-tridecane *at 0.125 ng* ($\chi^2 = 29.00$, df = 1, $N = 30$, $P > 0.001$) and 0.25 ng ($\chi^2 = 19.95$, df = 1, $N = 30$, $P > 0.001$; Figure 6B).

Discussion

The position of group members during a hunt is vital and generally mediated by acoustic or visual cues (Lang and Farine 2017). Here, we show that the group-hunting termite specialist *M. analis* uses short-chain hydrocarbons to communicate with nestmates over a short distance. This allows for a highly coordinated hunt that, to our knowledge, is unique among predatory ants like those in the genus *Leptogenys* spp and *Dorylus* spp.

Odor plume composition and detection

The results of the olfactometer assays show that volatiles mediate nontactile conspecific communication in workers of *M. analis*, suggesting that ants use volatiles to communicate their presence under group-related foraging conditions. In our study, no significant changes were observed in the responses of major and minor workers to the volatiles of living major and minor ants, suggesting that ant odors may play a generalist role in signal presence or absence of nestmates. Ants also preferred conspecific odors over odors from cues they commonly encounter during raids, such as termites in their galleries (Yusuf, Crewe, et al. 2014), indicating a greater sensitivity to detecting their own odors, especially as found in the current study for major workers. However, it is important to note that several factors may determine the quality and quantity of odors released from termites in a gallery, including the presence or absence of food, temperature, humidity, and composition of the microbial community in the gallery. Thus, although our results show differential sensitivity between major and minor workers to the odors released from the termites in their gallery and conspecific odors, further behavioral experiments are required to understand these interactions. Communication and maintaining social cohesion among workers in *M. analis* is vital due to their dietary specialization (termites), that is, highly coordinated group-raiding behavior and raid phases that change within minutes (Frank and Linsenmair 2017a; Yusuf, Gordon, et al. 2014). Hence, they rely on a sophisticated communication system using pheromones to achieve the maximum reward during foraging. Earlier studies have reported similar chemical communication in some ants. For example, the attraction of adults of the red imported fire ant *Solenopsis invicta* to volatiles from conspecific larvae (Glancey and Dickens 1988) and an odor-based queen–queen recognition system in the hairy panther ant *Neoponera villosa* (D'Ettore and Heinze 2005).

Analysis of volatiles showed that odors emitted by major and minor workers were identical, comprising primarily saturated hydrocarbons whose quantities were greater in majors than minors. Previously, we had used CHC profiles to group different ant colonies successfully (Yusuf et al. 2010). Interestingly, the ant odor plume does not seem to allow accurate nestmate recognition and is highly conserved across populations in Africa (Mozambique, Côte d'Ivoire, and Kenya), while the CHC profile varies greatly between these populations. We, therefore, believe that there was little evolutionary pressure for the odor plume to be used for nestmate recognition, but rather it is used as a conspecific recognition signal. During raids, contacts through direct interactions with nonnestmates are very rarely observed (pers. obs.), making it unnecessary to add complexity to the plume to achieve nestmate recognition. If the raiding group does indeed meet another colony, the normal CHC recognition profile should suffice for the colonies to identify nonnestmates.

The compound released in the highest proportion from the volatiles and CHC profiles of *M. analis* is *n*-undecane. Interestingly, *n*-undecane had previously been identified in the Dufour's gland secretions of M. analis and reported as a potential alarm pheromone (Longhurst et al. 1979). *n*-Undecane is also an alarm pheromone in some ant species like Camponotus pennsylvanicus, *Camponotus herculeanus* (Ayre and Blum 1971), *Camponotus obscuripes* (Fujiwara-Tsujii et al. 2006), and many Formicidae spp. (Verheggen et al. 2010). *n*-Pentadecane, another major component identified in the volatiles, is one of the major components in the Dufour's glands of the giant bull ant Myrmecia gulosa (Cavill and Williams 1967) and cape harvester ant *Messor capensis* (Brand and Mpuru 1993). These cross-generic similarities in the major alarm pheromone systems of ants suggest that they may have been biosynthesized early in the evolution of ant societies and have remained conserved over time.

However, to eliminate the possibility that the ants in our bioassays responded to an alarm rather than a task or communication pheromone, we tested different doses of *n*-undecane and those of *n*-tridecane, another alarm pheromone, respectively. In both tests, workers responded differently to different concentrations of the 2 compounds with preferences for these individual volatiles at low concentrations, but an alarm response was elicited in the ants at higher concentrations (Figure 6). Thus, odor-mediated behavior in this ant species appears to be concentration dependent and clearly suggests that odors may serve as task allocation cues, with odors released at concentrations above colony thresholds being perceived differently by different castes. Despite these findings, further research should look to test the other components identified in the volatiles, individually and as a blend to elucidate the role of the full spectrum of the volatiles released by workers of *M. analis*.

Odor plume benefits during raids

A constantly emitted odor plume by ants in raiding parties could be beneficial during raids. Previous studies have shown a clear formation within the raiding column, with ants even retaking a position at the front or tail of the column if displaced (Frank and Linsemair 2017b). Potential position-dependent odors within the column could be beneficial in identifying and maintaining column formation. The raid leader also halts the raiding party shortly before arriving at the hunting ground to gather the members of the column to raid as a coherent group. An increasing concentration of ant odor could be used as a cue to determine whether enough ants have gathered to start the next phase of the raid (Frank and Linsenmair 2017a, 2017b).

During the raid itself, the raiders recognize the onset of the return journey with the odor decreasing in concentration on the hunting site and the ants gathering and waiting at the starting point of the

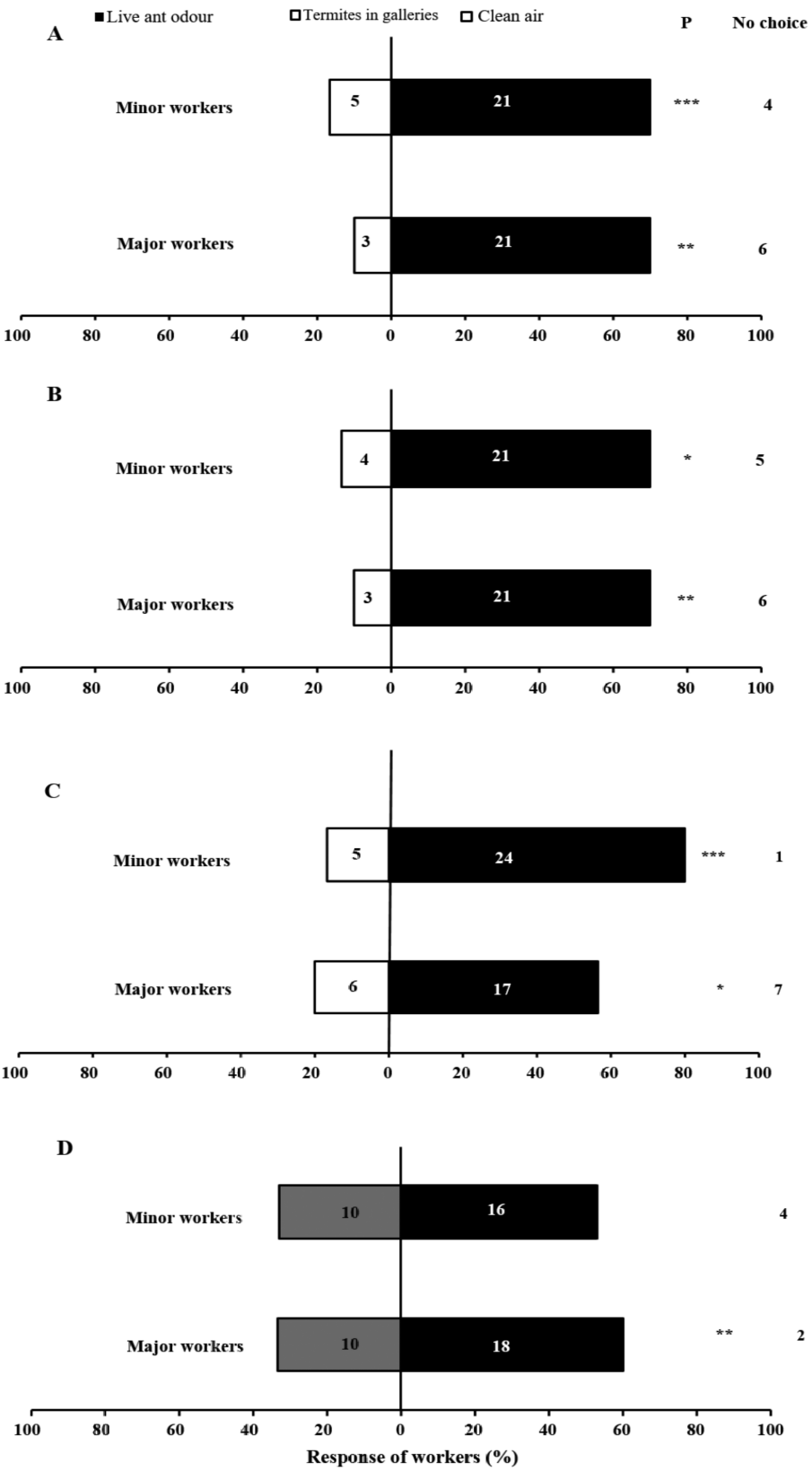

Figure 4. Preferences of minor and major workers of *M. analis* for odors of **(A)** mixed groups of ants (major and minor), **(B)** major workers, **(C)** minor workers against the control clean air (open bars), and **(D)** between odors from mixed workers and termites in galleries. Numbers within bars represent the number of ants making a choice, while numbers outside bars refer to ants that made no choice in the assay, $N = 180$, 90 each for major and minor workers in each treatment. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$).

Figure 5. Preferences of workers of *M. analis* for different concentrations of conspecific odors (12.5, 25, 50, and 100 ant hours) against the control odor clean air (open bars). Numbers within bars represent the number of ants making a choice, while numbers outside bars refer to ants that made no choice in the assays. $N = 160$, 40 replicates for each treatment, $^{***}P < 0.001$).

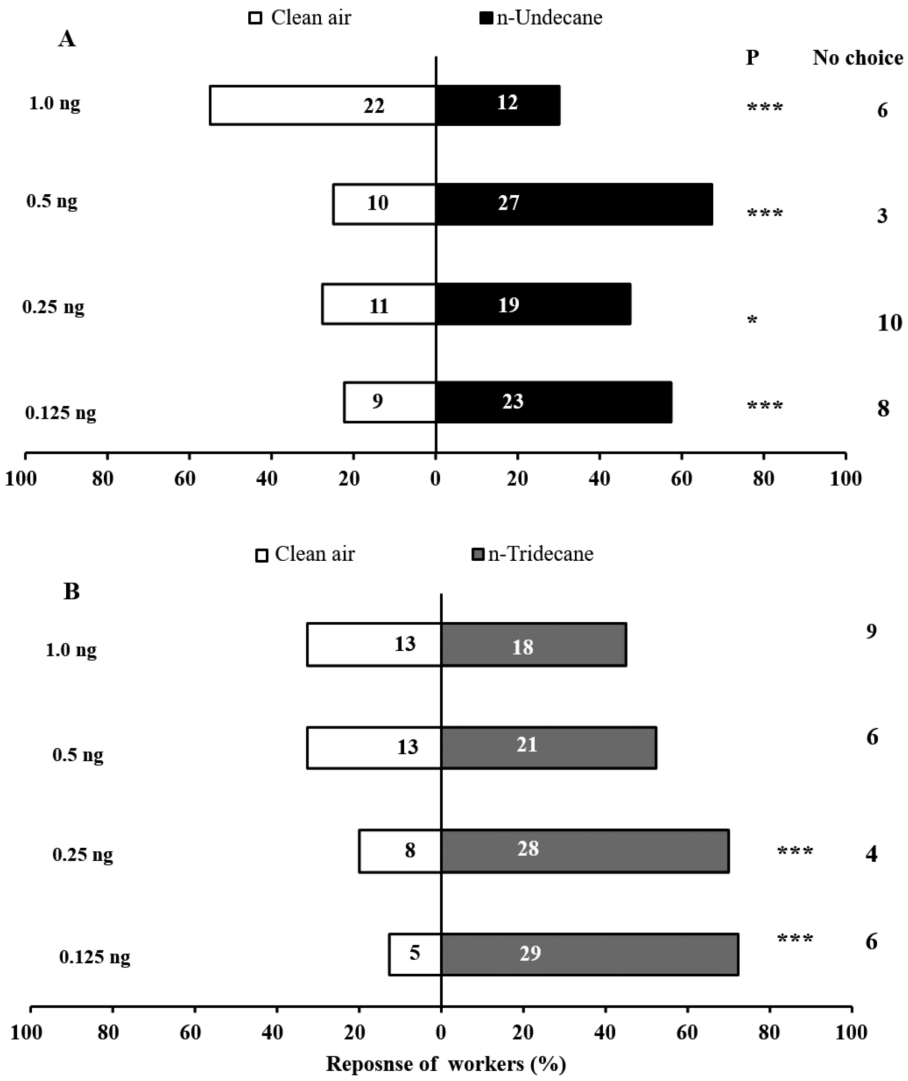

Figure 6. Responses of *M. analis* workers to **(A)** *n*-undecane *and* **(B)** *n*-tridecane *at 0.125, 0.25*, 0.5, and 0.1 ng compared to the control clean air (open bars). Numbers within bars represents the number of ants making a choice, while numbers outside bars refer to ants that made no choice, $N = 320$, 40 ants for each assay. Asterisks represent statistical significance ($^{*}P < 0.05$, $^{***}P < 0.001$).

raid. Ultimately, odors could explain the stark contrast in the behavior of injured ants in the presence or absence of nestmates even without direct contact. If nestmates are nearby, the injured ant stays mostly motionless or on its back; if nestmates are absent, the injured ant will immediately start the return journey to the nest (Frank et al. 2018).

Chemical communication in group-foraging ants

While group foraging is a common occurrence in social insects, it is rarely followed by such a complex coordinated hunt. Army ants for instance hunt in large groups comprising 100 000 individuals starting with one main trunk from the nest and branching out to cover the entire ground subduing prey as they encounter it and recruiting nestmates with secretions from their mandibular gland (Bruckner et al. 2018). This form of group foraging may not necessitate the use of an odor plume since it is relatively uncoordinated and differs from those of *M. analis*. First, army ants are in constant direct contact with one another during a raid, while *M. analis* workers at the hunting ground fan out into dozens of smaller "termite hotspot" hunting sites in an area of approximately 1 m^2 (Frank and Linsenmair 2017b). Second, foraging in army ants is devoid of clearly distinguishable hunting phases like coordinated recruitment, outward journey, duration at food source, and return journeys. Army ants generally leave the nest and subdue prey as they encounter them, this opportunistic hunting approach inhibits the development of a truly specialized hunting strategy with different phases as is the case in *M. analis* and necessitates less-specific coordination between nestmates to succeed. Lastly, the hunt is not as temporally constrained as it is in *M. analis*. In army ants, a foraging bout can last up to 12 h, while, in *M. analis,* it is often over after just 10 min (excluding travel time). A quick nontactile information transfer for the position of nestmates in such a short time window could be essential. Furthermore, considering the high cost of producing and maintaining a volatile odor plume, it might be too costly for longer periods of time but essential for precision and success during shorter raids.

Apart from the Dorylinae, the other predatory subfamily in ants is the Ponerinae, to which *M. analis* belongs. The vast majority of ponerines are solitary hunters in which there would be no necessity for such an odor plume. *Megaponera analis* clearly stands out with their large group-foraging strategy, continuous allometric size polymorphism, an unusually large colony size, and an ergatoid queen, all of which are army ant-like but unique adaptations for a ponerine species. While there are other ponerine species that hunt in groups, notably in the genus *Leptogenys* and *Neoponera*, in many cases, this is to subdue larger prey (like millipedes or Isopoda), where the benefit would be minimal, that is, restricted to local recruitment. There are some species of *Leptogenys* and *Neoponera* that show a similar hunting strategy as *M. analis* toward termites, albeit on a smaller scale. We expect an odor plume to have a similar benefit in these species as well. Further studies into the hunting behavior and chemical communication of these species could provide interesting leads on its evolution.

Conclusion

In this study, we identified 12 hydrocarbons and tested responses of ants to 2 previously identified components reported as alarm pheromones emitted by the ants. Moreover, the recorded EAG responses suggest that the different hydrocarbons may induce distinct receptor activity. We found behavioral response in *M. analis* to be both concentration and caste dependent.

Finally, we demonstrated odor-based communication in *M. analis* and its possible role in task allocation, thereby contributing to social cohesion. We propose that this odor-based short distance communication system might have evolved as an alternative to visual or acoustic communication in a group-hunting species with a high demand for quick differentiated and coordinated behaviors during their foraging phase.

Supplementary material

Supplementary material can be found at *Chemical Senses* online.

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Conflict of interests

The authors declare no conflict of interests.

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