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Chemical communication during foraging in the harvesting ants *Messor pergandei* and *Messor andrei*

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Abstract We combined behavioral analyses in the laboratory and field to investigate chemical communication in the formation of foraging columns in two Nearctic seed harvesting ants, *Messor pergandei* and *Messor andrei.* We demonstrate that both species use poison gland secretions to lay recruitment trails. In *M. pergandei*, the recruitment effect of the poison gland is enhanced by adding pygidial gland secretions. The poison glands of both species contain 1-phenyl ethanol. Minute quantities (3 µl of a 0.1 ppm solution) of 1-phenyl ethanol drawn out along a 40 cm long trail released trail following behavior in *M. pergandei*, while *M. andrei* required higher concentrations (0.5–1 ppm). *Messor pergandei* workers showed weak trail following to 5 ppm trails of the pyrazines 2,5-dimethylpyrazine and 2,3,5-trimethylpyrazine, whereas *M. andrei* workers showed no behavioral response. Minute quantities of pyrazines were detected in *M. pergandei* but not in *M. andrei* poison glands using single ion monitoring gas chromatography–mass spectrometry.

Keywords Column foraging · Harvester ants · 1-phenylethanol · Poison gland · Recruitment

Introduction

Among the several foraging strategies known in ants (see Hölldobler and Wilson 1990, 2009) column foraging in three Nearctic seed harvesting ants in the genus *Messor* (*M. pergandei*, *M. andrei*, *M. julianus*) is one of the most spectacular modes. Thousands (in *M. andrei*) and tens of thousands (in *M. pergandei*) of workers emerge from the nest and move along a narrow path away from the nest for distances of 3 to more than 40 m before they disperse in a foraging fan, where individuals leave the trail and forage independently, then return to the column after they have collected a food item (Went et al. 1972; Bernstein 1975; Wheeler and Rissing 1975). The direction taken by a column is determined at the beginning of each foraging bout. Columns always originate at the nest and direction may change between morning and evening and between subsequent days (e.g., Clark and Comanor 1973). Columns function to direct workers to harvesting sites while simultaneously avoiding neighbors (Ryti and Case 1988; Plowes et al. 2014). In *M. andrei*, foraging columns are more sedentary, with columns often taking the same direction for several successive foraging bouts. Columns may form trunk trails when they lead to a more stable resource (Plowes et al. 2013). In both *M. pergandei* and *M. andrei,* inter-colony aggression occurs when columns from neighboring colonies intersect (Wheeler and Rissing 1975; Brown and Gordon 2000).

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Mechanisms by which column foraging arise are poorly understood in *M. pergandei* and in the other two column foraging *Messor* species (*M. julianus*, *M. andrei*). Pilot tests by Blum (1974) indicated that *M. pergandei* workers follow trails laid with poison glands secretions, but Wheeler and Rissing (1975) claimed that there was 'no evidence of trail-marking' in the field. This paper reports our experimental analyses of the chemical communication mechanisms underlying group foraging in *M. pergandei* and *M. andrei*.

Methods

Animal collection and care

We collected foragers from seven colonies of *M. pergandei* and *M. andrei* for laboratory experiments. Workers of *M. pergandei* were collected at South Mountain Park, Phoenix, Maricopa County, AZ (33°19′51″N, 112°01′06″W; 435 m), while those of *M. andrei* were collected at Table Mountain Preserve, Auberry, Fresno County, CA (37°01′01″N, 119°35′04″W; 350 m). Each colony was divided into subgroups consisting of 300–1,000 workers, which were placed in large Plexiglas arenas (50 cm × 100 cm) with a 3 cm layer of plaster. Nest spaces (10 cm × 15 cm or 15 cm × 20 cm) in the plaster had glass lids covered with red acetate film. Ants were fed Bhatkar diet (Bhatkar and Whitcomb 1970), sugar water, and Rye grass seeds ad libitum. The plaster floor and nests were kept moist. Arenas were maintained in a room with a 12 h light/dark cycle at approximately 26 °C. Most workers remained in the nest, while small numbers (50+) left the nest to forage. Worker behavior was similar to that in the field, with workers forming columns and foraging during early morning and returning to the nest at night.

Behavioral assays

Laboratory experiments

We presented test groups with two artificial trails that consisted of glandular extracts or synthetic chemicals, using hexane as a solvent. Cardstock was placed on the arena floor, and trails were first drawn on the cardstock using a pencil, which provided a guide for placing chemical trails and observing the response. Chemical trails were drawn on the line using a microsyringe or hard wood applicator. The two trails originated from one point at the nest entrance, and then diverged by approximately 50° for 8 cm; the trails subsequently paralleled each other approximately 13 cm apart for 32 cm (total trail length of approximately 40 cm).

Preliminary results (Blum 1974) suggested that the poison gland was the source of trail recruitment chemicals, so we focused our efforts on this gland. We offered test groups of *M. pergandei* and *M. andrei* a choice between trails consisting of one gland equivalent (10 glands in 100 µl hexane, trail laid with 10 µl of extract) of poison gland versus a control (hexane only), Dufour gland, pygidial gland, or hindgut contents.

The pygidial gland contents, in which *n*-tridecane is the primary active component, also elicit an alarm-recruitment response in both *M. pergandei* and *M. andrei* (Hölldobler et al. 2013). Consequently, we tested if adding pygidial gland contents to the poison gland enhanced the recruitment response. Our experiments presented test groups with a choice between two sets of trail compounds: (1) one trail with poison gland extract, the other with hexane, and (2) one trail with poison gland plus pygidial gland extract, the other with hexane. Each test group was presented with both sets of trail compounds in random order; the second set of compounds was presented to the same test group after 1 h. These trials used 0.12 gland equivalent of poison gland secretions (5 µl of the following: 5 glands in 200 µl hexane) or 0.12 gland equivalent of poison gland secretion plus the contents of one pygidial gland.

We investigated whether *M. pergandei* and *M. andrei* could follow each other's trails by offering test groups a choice between either conspecific or non-conspecific trails laid with one poison gland equivalent. In smaller test groups (about 300 workers), we presented conspecific and non-conspecific trails simultaneously, in larger test groups (600–900 workers), we had to employ a sequential presentation to better quantify trail following behavior: *M. pergandei* and *M. andrei* test groups were presented with conspecific and non-conspecific poison gland secretions in alternative order, i.e., either conspecific first and non-conspecific second, or vice versa; the second set of compounds was presented to the same test group after 30–60 min.

The number of workers that walked from the nest entrance to the end of the trail within 1 min was our response variable in all of the above experiments. All data were analyzed using a paired *t* test and Wilcoxon Rank Sum test. Most of all the above mentioned experiments were conducted from 6:00 to 10:00, during the daily period of peak foraging activity. Some tests were conducted in the afternoon.

Field observations and experiments in M. pergandei

We tested whether workers of *M. pergandei* would follow poison gland extracts in the field. Experiments used a piece of white cardboard on which we had used a pencil to draw a 3 m trail, which was used to place artificial trails and observe the response. The cardboard was placed

so that the beginning of the artificial trail began within the nest yard, where workers had gathered but a column had not yet formed. Hexane extracts of poison gland secretions were applied to the 3 m line. One gland equivalent or less elicited trail following in the laboratory. In the field, we applied approximately 10 gland equivalents along the 3 m trail. All field observations were made at South Mountain Park between August 2010 and May 2012 from between 4:00 and 8:00. We video recorded commencement of foraging and trail following behavior in all laboratory and field experiments for subsequent analyses.

Chemical analyses

Messor pergandei workers used for chemical analyses were collected from South Mountain Park. Workers were chilled in the field and remained chilled until they were dissected in the laboratory. *Messor andrei* workers were transported from California to Arizona, where they were kept in the laboratory for up to several weeks prior to dissection.

Workers were dissected under water, and dissected poison glands were rinsed in distilled water, and then placed in hexane. The poison glands in *M. pergandei* were substantially smaller than those in *M. andrei*, therefore, we used 10 *M. pergandei* glands in 10 μl of ultrapure hexane (Sigma-Aldrich) and 7 *M. andrei* glands in 15 μl of ultrapure hexane (Sigma-Aldrich).

Aliquots of poison gland extracts (3 μl for *M. andrei*, 4 μl for *M. pergandei*) were analyzed using a Hewlett-Packard 5972 gas chromatograph–mass spectrometer (GCMS) fitted with a Supelco MDN-5 capillary column (30 m × 0.25 mm × 0.25 μm film thickness). Oven temperature was held at 40 °C for 2 min, then increased by 7 °C min^{-1}–250 °C and held for 8 min. Helium was used as the carrier gas (1 mL min^{-1}). Samples were introduced by splitless injection at 200 °C.

We also ran total ion chromatogram (TIC) and single ion monitoring (SIM) for poison gland contents of *M. pergandei* so as to detect minute quantities of pyrazines that were not detected using GCMS. Total ion chromatogram data were collected over a mass range of 50–300 at a rate of 1.78 scans per second using positive-ion electron ionization (70 eV). Total ion chromatogram peaks were identified by comparing mass spectra with National Institute of Science and Technology 05 Mass Spectral Library spectra. Single ion monitoring was used to detect trace levels of putative pheromones (Table 1). We confirmed retention times by running standards for known concentrations (1 ppm, 5 ppm) of three putative pheromone compounds: (1) trimethylpyrazine (TMP) (Sigma-Aldrich), (2) 2,5-dimethylpyrazine (DMP) (Sigma-Aldrich), and (3) 1-phenylethanol (1PE) (Sigma-Aldrich).

Table 1 Hexane extracts of *Messor pergandei* poison glands were subjected to single ion monitoring gas chromatography–mass spectrometry using the following mass fragments and retention times. Scan time was based on retention times of 3 μl of 5 ppm standards

Compound	Mass fragments	Time scanned (min)	Retention time (min)
1-Phenylethanol	79, 107	9.5–11	~10
2,3,5-Trimethylpyrazine	81, 122	8–9.5	8.6
2,5-Dimethylpyrazine	81, 108	5–8	~7

Table 2 Trail following behavior by workers of *Messor pergandei* and *Messor andrei* when offered a choice between a one gland equivalent trail consisting of conspecific poison gland contents versus a control (hexane only), Dufour gland, pygidial gland, or hindgut contents. Response is the number (mean ± 1 SE) of ants following the trail from the nest entrance to the endpoint in one minute

Test species	*n*	Response	
		Poison	Hexane
M. pergandei	5	50 ± 6	0 ± 0
			Dufour
	5	71 ± 14	0 ± 0
			Hindgut
	6	37 ± 7	0 ± 0
			Pygidial
	5	61 ± 13	0 ± 0
			Dufour
M. andrei	4	37 ± 9	0 ± 0
			Hindgut
	6	53 ± 10	0 ± 0
			Pygidial
	4	66 ± 17	0 ± 0

Results

Behavioral assays

The poison gland was determined to be the source of recruitment trail pheromone for both *M. pergandei* and *M. andrei* (Table 2). In *M. pergandei*, secretions from the poison gland elicited significantly greater trail following as compared to hexane controls (paired *t* test, $t = 8.30$, $P = 0.001$, $n = 5$), pygidial gland extracts ($t = 4.73$, $P = 0.009$, $n = 5$), Dufour gland extracts ($t = 4.93$, $P = 0.008$, $n = 5$), and hindgut contents ($t = 5.39$, $P = 0.003$, $n = 6$). Similar results were obtained with *M. andrei* (Table 2; poison vs. pygidial: $t = 3.91$, $P = 0.03$, $n = 4$; poison vs. Dufour: $t = 4.21$, $P = 0.024$, $n = 4$; poison vs. hindgut: $t = 5.11$, $P = 0.004$, $n = 6$).

These results were also documented for *M. pergandei* in the field, where trail following was induced by a three meter long trail laid with 10 poison gland equivalents

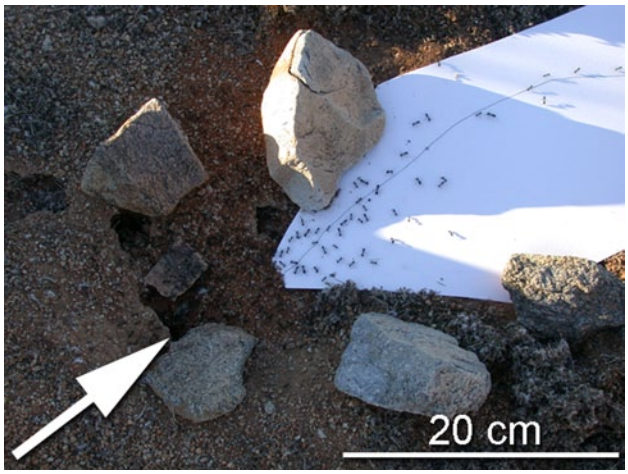

Fig. 1 An extract of poison gland secretion (one gland equivalent in hexane) placed on the line on the cardstock induced trail following by *Messor pergandei* workers in the field. The nest entrance is at the tip of the *white arrow* (photograph by N. Plowes)

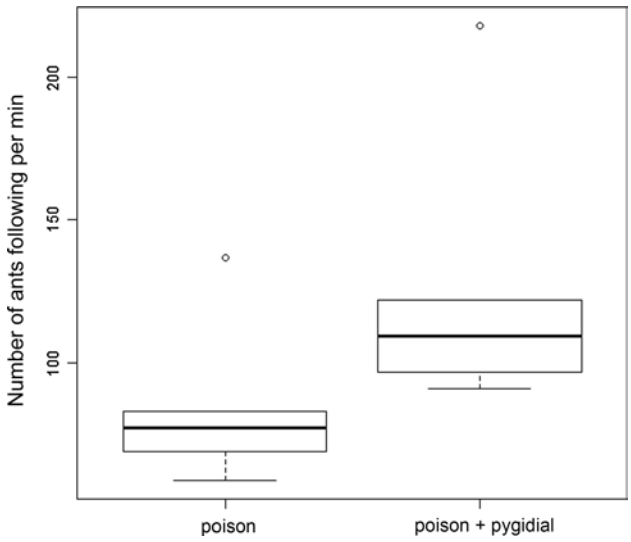

Fig. 2 Trail following behavior by *Messor pergandei* to a one gland equivalent trail consisting of poison gland or poison gland plus pygidial gland contents from *M. pergandei*. Response was measured as number of ants following the trail from the nest entrance to the endpoint of the trail in 1 min, and was significantly higher in tests with poison plus pygidial gland contents (Wilcoxon Rank Sum, $W = 5$, $P = 0.04$, $n = 5$). *Box* and *whisker plots* illustrate: median (*thick black*), upper and lower quartiles (*box*), maximum and minimum (*whiskers*), outliers (*open circles*)

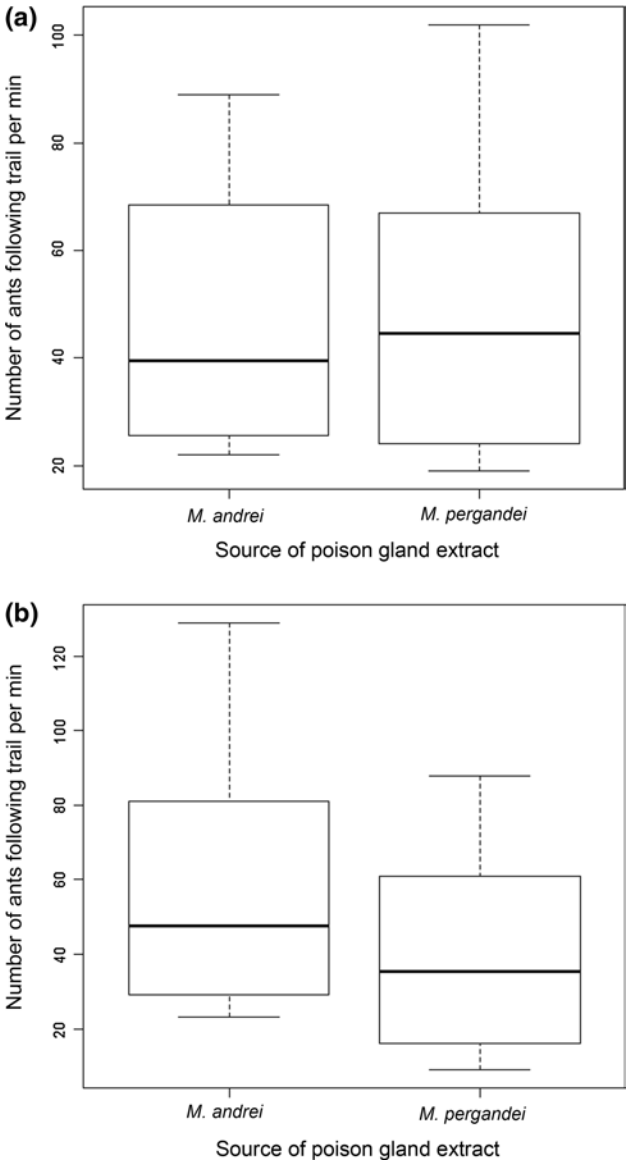

Fig. 3 Trail following behavior to a choice of either one gland equivalent trail consisting of conspecific or of non-conspecific poison gland contents for (**a**) *Messor pergandei* (*t* test, $t = -0.13$, $P = 0.90$, $n = 8$) and (**b**) *Messor andrei* (Wilcoxon rank sum, $W = 135$, $P = 0.09$, $n = 14$). Response was measured as number of ants following the trail from the nest entrance to the endpoint of the trail in one minute. *Box* and *whisker plots* illustrate: median (*thick black*), upper and lower quartiles (*box*), maximum and minimum (*whiskers*)

(Fig. 1). Note, however, that artificial trails only elicited trail following when placed at the nest entrance before a column had started to form. Workers followed the trail for more than 3 m.

When foraging commenced, both in the field and laboratory, *M. pergandei* workers exhibited rapid jerking runs at the nest entrance or inside the nest, and subsequently, additional ants emerged from the nest. We had the impression that jerking ants stimulated nestmates to leave the nest. A recent study demonstrated that such alarm-recruitment effects can be elicited in these two species by adding pygidial gland contents, the main active component of which is *n*-tridecane (Hölldobler et al. 2013). Our experiments on *M. pergandei* demonstrated significantly higher trail following (mean increase of 49 %) on trails laid with a mixture of poison and pygidial gland extracts as compared

to only poison gland extracts (Wilcoxon Rank Sum, $W = 5$, $P = 0.04$) (Fig. 2). These and other tests with poison gland secretions indicated that an increased concentration of poison gland secretions on the trail does not necessarily lead to a stronger behavioral response, but adding pygidial gland secretions appeared to modulate the response threshold to the trail pheromone.

Our experiments on response to conspecific and non-conspecific poison gland contents demonstrated that workers of *M. pergandei* and *M. andrei* responded similarly to trails laid with poison gland extracts of both species (*M. pergandei*: *t* test, $t = -0.13$, $P = 0.90$, $n = 8$; *M. andrei*: Wilcoxon rank sum, $W = 135$, $P = 0.09$, $n = 14$; Fig. 3).

Column formation

During summer months, *M. pergandei* colonies foraged from ~4:30 to 8:00, depending on temperature. In winter, foraging occurred during the middle of the day when temperatures were above 13 °C. There was a predictable sequence of phases in the formation of foraging trails: initiation, extension, marching, harvesting, and termination.

During initiation, ants gathered at the surface of the mound (nest yard), followed by a small number of ants (1–5) leaving the nest yard and moving short distances (10–20 cm) away from the main platform. We conducted detailed scans around nests prior to commencement of column formation, but we never observed behaviors suggesting that columns were initiated by scouts (ants returning to the nest). The extension phase was marked by numerous ants following one to few individuals who had begun to walk away from the nest yard (Video 1); follower ants moved slowly in a circuitous manner, advancing about 10–30 cm, then circling back. Once the trail was 1–2 m long, ants exiting the nest behaved in a more determined manner. They lined up to join the column and moved in a straight line along it, and individuals touched their gasters to the surface of the ground (Video 2). The harvesting phase began when workers at the end of the column dispersed in the so-called foraging fan, walking in loops and circles, searching for seeds. After a food item was collected, individuals returned to the column along a relatively straight line, then ran rapidly back to the nest. The harvesting phase lasted 1.5–2 h, depending on temperature. The termination phase was marked by a sudden decrease in foragers exiting the nest and a rapid influx of returning foragers. This was the most rapid of the phases, lasting 10–15 min, although there was considerable variation between colonies and it also appeared to be temperature-dependent. This phase ended after all foragers had returned to the nest, leaving a few ants guarding the nest entrance (see also Rissing 1988).

Chemical analysis and identification of trail pheromone

GCMS analysis of *M. andrei* poison glands revealed several volatile substances, whose mass spectra and retention time matched those of (1) 1-phenylethanol (1PE); (2) tridecane; (3) E2-hexadecen-1-ol; (4) pentadecane; (5) hexadecanoic acid; (6) oleic acid; (7) 2-propenoic acid, [3-(4-methoxyphenyl)-, 2-ethylhexyl ester (CAS #5466-77-3)] (Fig. 4a). Hexane extracts of poison glands from *M. pergandei* run with TIC GCMS indicating small quantities of (1) 1PE (Fig. 4b). Single ion monitoring (SIM) of *M. pergandei* poison gland extracts showed an explicit peak consistent with retention time for 1PE (Sigma-Aldrich).

The fact that the closely related myrmicine ant *Aphaenogaster* (=*Novomessor*) *cockerelli* follows poison gland secretions containing 1PE (Fig. 5) (Hölldobler et al. 1995) led us to test whether the two *Messor* species also used 1PE. We laid 5 ppm 1PE trails (15 ng of 1PE in 3 μl of

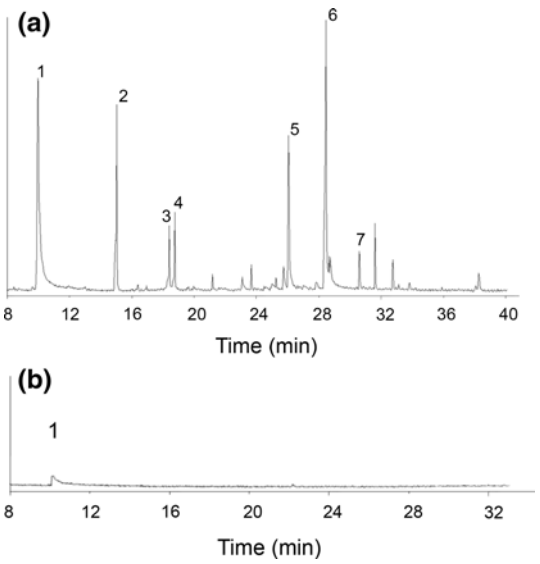

Fig. 4 Total ion chromatogram gas chromatograph–mass spectrophotometer chromatogram for the contents of: (**a**) a 3 μl sample of 10 *Messor andrei* poison glands dissolved in 15 μl of hexane, and (**b**) a 4 μl sample of 10 *Messor pergandei* poison glands dissolved in 10 μl of hexane. The *peaks* represent: *1* 1-phenylethanol; *2* tridecane; *3* E2-hexadecen-1-ol; *4* pentadecane; *5* *n*-hexadecanoic acid; *6* oleic acid; *7* 2-propenoic acid, [3-(4-methoxyphenyl)-, 2-(ethylhexylester)]

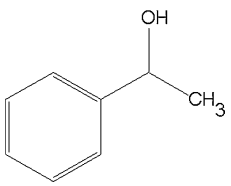

Fig. 5 Chemical structure of 1-phenylethanol (1PE), which is the primary trail pheromone in *Messor pergandei* and *Messor andrei*

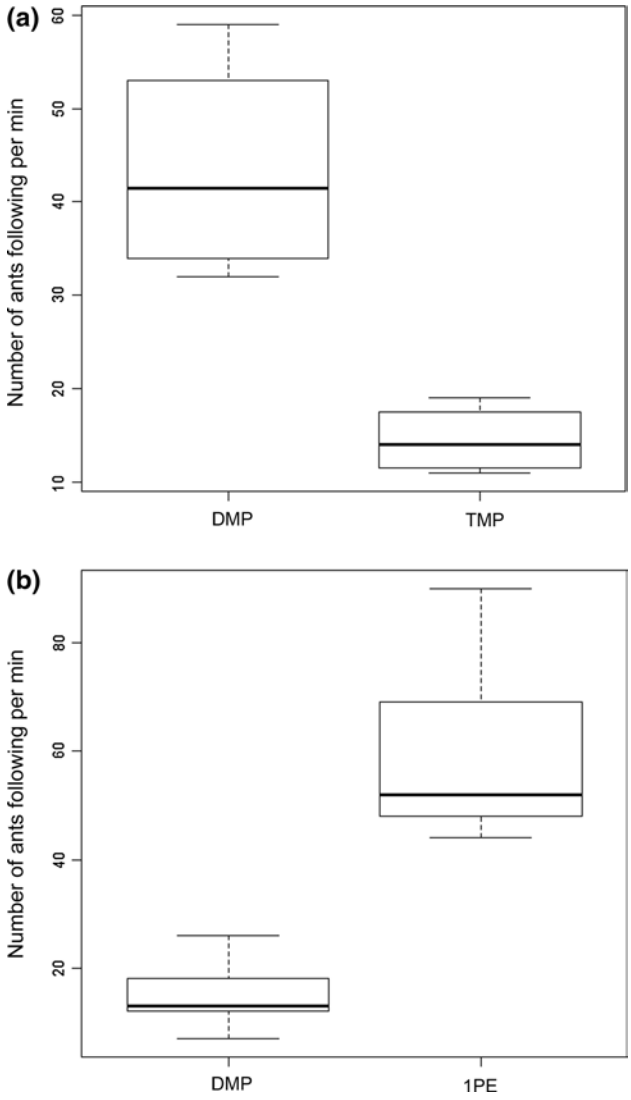

Fig. 6 Trail following behavior by *Messor pergandei* to two pyrazines: 2,3,5-trimethylpyrazine (TMP), and 2,5-dimethylpyrazine (DMP). Test groups were offered a choice between **a** DMP and TMP (*t* test, $T = 4.6$, $P = 0.02$, $n = 4$) or **b** DMP and 1PE (Wilcoxon rank sum, $W = 0$, $P = 0.008$, $n = 5$). Response was measured as number of ants following the trail from the nest entrance to the endpoint of the trail in 1 min. *Box* and *whisker plots* illustrate: median (*thick black*), upper and lower quartiles (*box*), maximum and minimum (*whiskers*)

hexane) along the bottom of plaster nests containing worker groups of *M. andrei* and *M. pergandei*. Both species showed a strong trail following response (example in *M. pergandei*: Video 3). Further tests demonstrated that *M. pergandei* workers showed clear trail following with as little as 3 µl of a 0.1 ppm 1PE solution, while *M. andrei* require higher concentrations of 1PE (weak trail following at 0.5 ppm, clear trail following at 1 ppm).

Although 1PE is the major trail pheromone for both *Messor* species, we want to point out some puzzling results that we obtained in pilot experiments testing three pyrazines (3-ethyl-2,5-dimethylpyrazine [EDMP], 2,3,5-trimethylpyrazine [TMP], and 2,5-dimethylpyrazine [DMP]) that function as trail pheromones in several myrmicine ants (reviewed in Morgan 2009). *Messor andrei* (data not shown) did not show trail following to any of these three pyrazines, whereas *M. pergandei* exhibited clear trail following to DMP and TMP (Fig. 6a), but not to EDMP (data not shown). In choice tests with DMP and 1PE, the latter was always significantly more effective as a trail pheromone (Wilcoxon rank sum, $W = 0$, $P = 0.008$, $n = 5$) (Fig. 6b). In all experiments, we used 3 µl of 5 ppm hexane solutions of the tested compounds. No pyrazines were detected in TIC GCMS analysis of *M. pergandei* but the SIM GCMS analysis indicated ions consistent with trace quantities of DMP and TMP. No ions consistent with any of these pyrazines were found in *M. andrei*.

Discussion

The harvesting ant genus *Messor* consists of 113 described species (Bolton 2013), of which nine are found in North America. Three species of Nearctic *Messor* (*M. pergandei*, *M. andrei*, *M. julianus*) are 'group-foragers', where individuals coordinate foraging efforts by forming trunk trails and foraging columns, and the remaining six species are solitary or individual foragers (reviewed in Johnson 2001; Plowes et al. 2013).

The trail pheromone of both column foragers *M. pergandei* and *M. andrei*, is 1PE, which is found in the poison gland. The same compound has previously been identified in the poison gland of *Aphaenogaster cockerelli* (previously *Novomessor*) where it serves as a signal in group recruitment (Hölldobler et al. 1995). These results lend further support to the supposition that Nearctic *Messor* are more closely related to a group of Nearctic *Aphaenogaster* than to species of Palearctic *Messor* (Bennett 2000; Ward 2005; Moreau and Bell 2013). The sensitivity to 1PE in *M. pergandei* is ten times higher than in *M. andrei*. This may also be reflected in the much smaller poison glands and the extremely small amounts of 1PE detected by GCMS in *M. pergandei*. 1PE has only been described as a recruitment pheromone in these three ant species, but is known as an attractant in scarab beetles (Vuts et al. 2010), and phorid flies (Kamm et al. 1987).

In both column foragers studied, *n*-tridecane, the major compound in large pygidial gland reservoirs of *M. pergandei* and *M. andrei* (Hölldobler et al. 2013), appears to modulate the response of workers to trail pheromone (1PE); it increased trail following by almost 50 % in *M. pergandei*. Presence of large pygidial gland reservoirs is restricted to the three column foraging Nearctic *Messor* species (*M.*

pergandei, *M. andrei*, *and M. julianus*) (Hölldobler et al. 2013). We hypothesize that pygidial gland secretions are released by leader ants during column initiation because workers following the leaders do not follow narrow trails (see workers following 1PE trail at the beginning of Video 3). The individuals following leaders may be spaced 3 cm or more apart, with the column 5–15 cm wide. This large pheromone 'headspace' is probably the result of aerosolized pheromone, rather than pheromone applied to the substrate.

We also detected tridecane in the poison gland of *M. andrei*, however, we do have to be cautious about this finding. It might be a contamination of pygidial gland secretions, because it is almost impossible to dissect the poison gland without accidentally rupturing the reservoir of the pygidial gland.

In Palearctic *Messor* species, the trail recruitment pheromone originates in the Dufour gland (confirmed in: *M. barbarus*, *M. lusitanicus*) (reviewed in Plowes et al. 2013). The biologically active components of Dufour or poison glands have not been elucidated for Palearctic *Messor* species. The most abundant and common compounds found in Dufour glands of Palearctic *Messor* include straight chain hydrocarbons, such as pentadecane, tridecane, and heptadecane (reviewed in Plowes et al. 2013). The poison glands of several Palearctic *Messor* species contain anabasine and anabaseine (reviewed in Plowes et al. 2013). In general the poison glands of Palearctic *Messor* species contain a wide variety of compounds, but only in *M. bouvieri* has 3-ethyl-2,5-dimethylpyrazine (EDMP) been identified to elicit some trail following behavior (Jackson et al. 1989). In our comparative tests with *Messor barbarus*, we also noticed some trail following behavior when trails drawn with crushed poison glands were presented to the ants, however, explicit trail following was only released by trails drawn with Dufour gland secretions.

While we have confirmed that trail pheromone along the length of the column maintains recruitment to a foraging fan and pygidial gland secretions can initiate the exodus from the nest area, choice of column direction, length of column, and timing of column termination, are still unknown. Temperature is likely to be an important trigger for both initiation and column termination, with activity limited between ~13 and 40 °C (Tevis 1958; Bernstein 1974; Gordon 1978; Rissing 1982). In the winter months, workers gather and warm on the nest yard, becoming more active and commencement of the column takes place when sunlight directly hits the nest. Light levels may factor in the hot summer months, because colonies often begin foraging in the morning during astronomic twilight (before sunrise). Wheeler and Rissing (1975) proposed that trails established by small numbers of workers at dusk allow colonies to forage during cooler night temperatures in the summer months. Column termination is precipitated by high surface temperatures (+40 °C), most likely representing the physiological limitation of workers (Bernstein 1974; Gordon 1978; Rissing 1982).

The direction chosen by foraging *M. pergandei* colonies has been the subject of many studies (reviewed in Plowes et al. 2013). Simulation models based on the behavior of a population of *M. pergandei* colonies demonstrate that a random choice of direction is inconsistent with behavior of field colonies (Plowes et al. 2014). Results suggest that column direction is chosen in response to the space use of neighbors allowing individual colonies to maximize resource acquisition (see also Ryti and Case 1988). The mechanisms by which colonies choose a foraging direction are still unknown. Workers could choose (or avoid) the direction with the highest concentration of pheromone residue remaining from the previous bout. Highly motivated ants may remember the direction of their foraging success, or aggressive encounters with conspecific neighbors. Modified behavior, as a result of experience with seed handling, has been demonstrated to last up to 7 days in *M. pergandei* (Johnson et al. 1994). We did not observe reconnaissance by scouting ants in *M. pergandei*. This is in contrast to other species that use group foraging, or group raiding strategies, such as *Pogonomyrmex* (Hölldobler 1976; Greene and Gordon 2007), *Acromyrmex* (Lopes et al. 2004), and *Rossomyrmex* (Ruano and Tinaut 1999). However, some chemical recruitment away from the column may occur when dense seed patches have been discovered by individual foragers in *M. pergandei* and this might be even more common in *M. andrei* (unpublished observations).

The genus *Messor* is of particular interest, because, like *Pogonomyrmex*, it has species displaying multiple different foraging strategies that range from individual to group foraging. There may be multiple solutions to the general problem of harvesting seeds from a central nest location, but we also expect to find examples of behavioral convergence. For example, individually foraging *Pogonomyrmex* species, when faced with a bonanza food resource, can recruit nestmates using secretions from poison glands (Hölldobler and Wilson 1970; for a review see Johnson 2000). The pheromones secreted by the poison glands of both individually foraging and group foraging *Pogonomyrmex* are pyrazines (3-ethyl-2,5-dimethylpyrazine [EDMP], trimethylpyrazine [TMP], and 2,5-dimethylpyrazine [DMP]) (Hölldobler et al. 2001). Do individually foraging *Messor* species use the same trail compound (1PE) as the group foraging species? The only described occurrence of pyrazines in *Messor* poison glands is in the Palearctic species *M. bouvieri* (Jackson et al. 1989).

Trace quantities of pyrazines were observed in SIM analyses of *M. pergandei* poison gland, and

workers followed trails laid with DMP and TMP, but not with EDMP, but this trail following response was considerably less explicit when compared with that to 1PE trails. As the presence of pyrazines in the poison glands of myrmicine ants is quite common (Morgan 2009), their total absence in *M. andrei* and the extremely small trace amounts in *M. pergandei* were unexpected. Perhaps the use of 1PE as trail pheromone in *M. andrei* and *M. pergandei* is a special adaptation to column foraging in Nearctic *Messor* species, and pyrazines occur in the less-derived individually foraging species of Nearctic *Messor*. Pilot tests have shown that such species (for example *M. smithi*) do recruit to dense seed falls, and they follow trails drawn with poison gland secretions. It will be interesting to see whether in such species, pyrazines serve as trail pheromones. If this is the case, we can postulate that the trace amounts of pyrazines and the response to trails drawn with pyrazines in *M. pergandei* are plesiomorphic traits, which have been lost in *M. andrei*.

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