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RAPID COMMUNICATION

Solitary Foraging in the Ancestral South American Ant, *Pogonomyrmex vermiculatus*. Is it Due to Constraints in the Production or Perception of Trail Pheromones?

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Abstract Several North American species of *Pogonomyrmex* harvester ants exhibit group foraging, whereas South American species are exclusively solitary foragers. The composition of the secretions of the poison and Dufour glands in the South American species, *Pogonomyrmex vermiculatus*, were analyzed, and the secretions and their components were tested as trail pheromones in laboratory bioassays. The major compounds in the poison gland were the alkylpyrazines, 2,5-dimethylpyrazine, 2,3,5-trimethylpyrazine, and 3-ethyl-2,5-dimethylpyrazine. The Dufour gland contained five alkanes, from tridecane to heptadecane, with pentadecane being most abundant. In behavioral bioassays, poison gland extracts and the mixture of pyrazines produced a trail pheromone effect, whereas the Dufour gland extracts and the alkanes had no effect on ant locomotion. We conclude that group foraging in *P. vermiculatus* does not arise from the inability to produce or detect possible pheromones, but rather, from physiological and/or ecological factors.

Keywords Harvester ants · Trail pheromones · Alkylpyrazines · Trimethylpyrazine · Pentadecane · Behavioral bioassays

Introduction

Harvester ants of the genus *Pogonomyrmex* are common in arid and semiarid habitats from southern Canada to the southern extreme of Tierra del Fuego (Taber, 1998). Phylogenetic reconstructions of the genus *Pogonomyrmex* based on morphological, chromosomal, ecological, and behavioral characters show the ancestral nature of the South American species (Taber, 1998). They are exclusively solitary foragers, in contrast to the North American species, some of which exhibit group foraging (a derived character) and generate and use trails for the exploitation of food patches (Johnson, 2001). In this latter group, group

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foraging behavior with recruitment of individuals is mediated by semiochemicals emitted from the poison and Dufour glands (Hölldobler et al., 2001). The major compounds secreted by the poison gland of species from North America are alkylpyrazines (Hölldobler et al., 2001), whereas the Dufour gland contains many types of compounds such as alkanes, alkenes, esters, terpenes, ketones, and aldehydes (Hölldobler et al., 2004). In this work, we tested whether the solitary foraging behavior of an ancestral species is due to its inability to produce or to detect chemical signals involved in group foraging. In the South American species, *Pogonomyrmex vermiculatus*, we identified the chemicals produced by the poison and Dufour glands and evaluated the effect of extracts of both glands and the chemicals contained therein on the locomotion behavior of the ants in laboratory bioassays.

Methods and Materials

Sample Collection Forager workers from five colonies were collected during January 2005 in Las Chinchillas National Reserve (31°30′ S, 71°06′ W). The ants were held in acrylic boxes and transported to the laboratory, where they were maintained according to standard rearing conditions (Hölldobler and Wilson, 1990).

Thirty worker ants of *P. vermiculatus* were killed by freezing (−18°C for 5 min). The poison and Dufour glands were separately dissected from each individual under distilled water by using a stereomicroscope. The individual glands were sealed in soft glass capillaries, which were introduced directly into the injector port of a gas chromatograph by using a solid sample injector as described by Morgan (1990).

GC-MS Analysis of Samples The analyses of compounds contained in the glands were carried out on an HP-5890 gas chromatograph coupled to an HP-5972 mass selective detector with an integrated data system. Ionization by electron impact (70 eV) was carried out at 280°C. The GC was equipped with a SPB-5 capillary column (30 m×0.25 mm ID, Supelco, Deerfield IL, USA). Helium was used as carrier gas at 2 ml/min. The capillaries containing the glands were heated in the injector port to 180°C for 3 min before crushing them in the solid sample injector. The oven temperature was held at 30°C for 2 min, then increased to 250°C at 7°C/min, then held at 250°C for 5 min. The presence or absence of a given compound in the chromatographic profile of each individual gland was determined by comparison of mass spectra with a library database and was confirmed by injection of authentic standards and comparison of Kovats indexes. This procedure was followed for alkanes, 2-tetradecanol, 2,5-dimethylpyrazine (DMP), and 2,3,5-trimethylpyrazine (TMP) (Aldrich Chem. Co.), and for 3-ethyl-2,5-dimethylpyrazine (EDMP) (Carlos Cramer Productos Aromáticos S.A.C.I., see below for details). When standards were not available (heptadecene), tentative identification was based on comparison of the mass spectrum with a library mass spectrum and reported Kovats index values. Identifications were considered positive if the similarity index between experimental and library mass spectra was higher than 90% and if Kovats indexes did not differ by more than 5 U (differences were typically less than 3 U). In the case of ethyldimethylpyrazine, where mass spectra are similar between isomers, an authentic standard that contained 3-ethyl-2,5-dimethylpyrazine and 2-ethyl-3,5-dimethylpyrazine was analyzed. These two compounds produced well-separated peaks, and the peak with the lower Kovats index, which was identified as 3-ethyl-2,5-dimethylpyrazine (E.D. Morgan, personal communication), coincided with the peak in the

samples analyzed. To quantify the pyrazines and alkanes present in single glands, calibration lines were generated with serial dilutions of authentic standards.

Bioassays with Gland Extracts Poison and Dufour glands were dissected from 40 worker ants. All glands of the same type were deposited in a sample vial containing 1.0 ml hexane, crushed, and filtered to produce extracts. Behavioral tests were performed in a Y-shaped Plexiglass olfactometer with 5×5 cm cross-section, 40-cm long stem, and 15-cm long branches at an angle of 30°. The floor of the olfactometer was lined with filter paper. Chemical trails extending from the base of the stem to the end of the branches were produced with 25 μl of gland extract (one-gland equivalent, treatment trail) and 25 μl of solvent (hexane, control trail). In the stem, chemical trails were drawn along imaginary lines 1.25 cm distant from the walls; in the branches, chemical trails were drawn along the center of the floor. Twenty-five seconds after application of the extracts and solvent, a test ant was liberated at the base of the stem. The first choice of arm was recorded, and the time allocated to this first preference was determined for each test ant. Twenty-four replicates for the poison gland treatment and 26 replicates for the Dufour gland treatment were performed. Additional bioassays were performed with one-gland equivalent of mixtures of the three pyrazine standards (45 ng, 40 replicates) and the five alkane standards (675 ng, 34 replicates), in the same proportions as those found in the glands. The arena was thoroughly cleaned with ethanol between replicates, and the filter paper liner was replaced. Data on the first choice of arm of the Y-olfactometer were analyzed by using a Chi-square test with one degree of freedom and Yates' correction factor; values for residence time in the first arm chosen by the ants were compared with *t*-tests for independent samples (Statistica 6.0 software). For both statistical tests, $\alpha=0.05$.

Results

The poison glands of *P. vermiculatus* contained a mixture of three alkylpyrazines: 2,5-dimethylpyrazine (DMP), 2,3,5-trimethylpyrazine (TMP), and tentatively identified 3-ethyl-2,5-dimethylpyrazine (EDMP). TMP was most abundant (67.7±3.1%, mean ± standard error), followed by DMP (20.1±4.2%), and EDMP (12.2±2.6%). The Dufour glands contained mainly five alkanes (96.9±0.6%: tridecane, tetradecane, pentadecane, hexadecane, and heptadecane), traces of 2-tetradecanol (0.1±0.01%), and a heptadecene (0.1±0.01%). Pentadecane was by far the major component (95.2±0.6%), followed by heptadecane (2.3±0.3%), tetradecane (1.4±0.2%), tridecane (0.9±0.2%), and hexadecane (0.2±0.02%).

In choice bioassays, significantly more worker ants went into the olfactometer arm containing the poison gland extract than the solvent control ($\chi^2=4.26$, $N=15$, $P<0.05$; Fig. 1a), and foragers spent more time in the arm with the poison gland extract than in the arm with the hexane control ($t=2.62$, $df=28$, $P<0.05$; Fig. 1c). This pattern was confirmed in tests that used authentic pyrazine standards, with ants preferring the treatment over the control arm ($\chi^2=4.32$, $N=28$, $P<0.05$; Fig. 1b). Ants also spent more time in the treatment than in the control arm ($t=2.93$, $df=54$, $P<0.05$; Fig. 1d). In contrast, ants showed no preference for the arm with Dufour gland extract (treatment=10, control=5, nonresponders=11; $\chi^2=1.06$, $P=0.30$), and spent equivalent amounts of time in the Dufour gland extract and hexane control arms (252±62 vs. 210±72 sec in the arm containing Dufour gland extract and solvent, respectively; $t=0.44$, $df=28$, $P=0.66$). Experiments with alkane standards confirmed these results. Thus, ants showed no preference for the arm with the alkane mixture vs. solvent

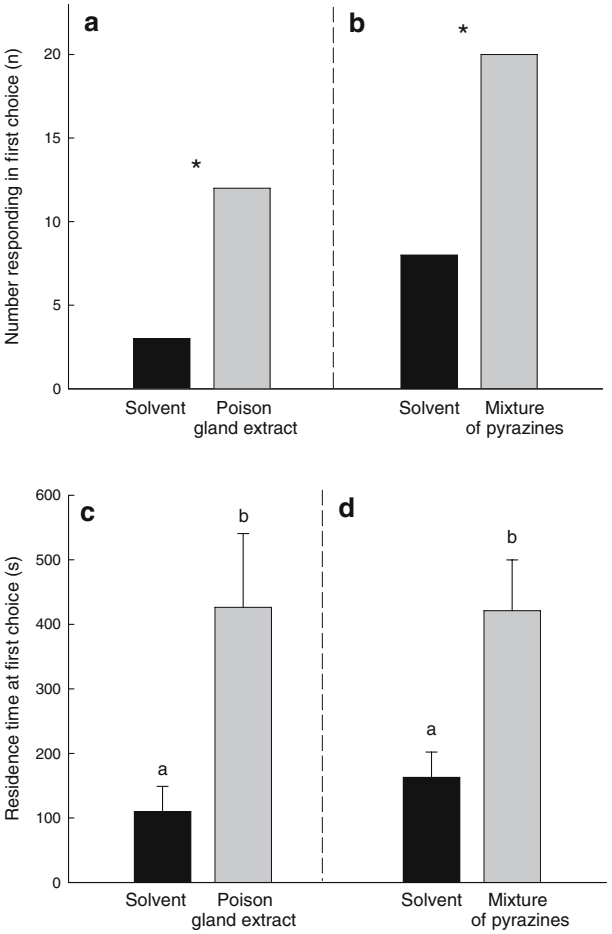

Fig. 1 **a**,**b** First arm choice of *Pogonomyrmex vermiculatus* workers exposed in a Y-olfactometer to different treatments vs. hexane solvent controls (*$P<$ 0.05, Chi-square test). **c**,**d** Time allocated to the arm of the first choice (values are means ± standard error; different letters above bars indicate significant differences in a *t*-test for independent samples)

(treatment=14, control=8, nonresponders=12; $\chi^2=1.14$, $P=0.28$) and spent similar amounts of time in the treatment and control arms (283±38 vs. 290±46 sec in the arm containing the mixture of alkanes and solvent, respectively; $t=0.13$, $df=42$, $P=0.89$).

Discussion

In group-foraging North American species of *Pogonomyrmex*, poison gland secretions constitute short-lived signals (20 to 30 min) allowing ants to recruit nestmates and move long distances towards food patches along trunk trails (see references in Hölldobler et al., 2001). These secretions contain pyrazines, which have been identified as trail pheromone components in several genera of the subfamily Myrmicinae (*Aphaenogaster*, *Atta*, *Eutetramorium*, *Manica*, *Messor*, *Myrmica*, *Pheidole*, *Pogonomyrmex*, and *Tetramorium*) (Hölldobler et al., 2001, and references therein). The poison gland of *P. vermiculatus* contained ca 45 ng pyrazines per gland, which is within the range reported for other

Pogonomyrmex species (30–200 ng per gland) (Hölldobler et al., 2001). Furthermore, our bioassays (Fig. 1 a–d) suggest that compounds present in the poison gland of *P. vermiculatus* can function as trail pheromones. Finally, the preference of treatment (poison gland extract or mixture of pyrazines) over control was ca 3:1 in the first choice of arm and also in the residence time. These proportions are comparable with the strength of recruitment behaviors elicited by pyrazines in group foraging *Pogonomyrmex barbatus* (ratio ca 4:1 for treatment vs. control; Hölldobler et al., 2001).

The secretions from the Dufour gland of group-foraging North American *Pogonomyrmex* serve as long-lasting orientation signals (on the order of hours) and allow continuous reinforcement of the trail leading to the foraging site with chemical signals specific for each colony (Hölldobler et al., 2004, and references therein). Hydrocarbons have been reported as the principal compounds secreted by the Dufour gland in the genus *Pogonomyrmex* (Hölldobler et al., 2004). Pentadecane, the major compound present in *P. vermiculatus*, has also been reported as the major compound in the Dufour glands of *Pogonomyrmex occidentalis* and *Pogonomyrmex salinus* (Billen et al., 1987, do Nascimento et al., 1993), two group-foraging species. However, neither the Dufour gland extract nor the reconstructed hydrocarbon mixture contained therein elicited changes in the locomotion of *P. vermiculatus* in relation to controls. This lack of effect may be due to the fact that *P. vermiculatus* only uses food patches situated near the nest (at distances <5 m, H. Torres-Contreras, personal observation), and hence, the reinforcement of trails with chemical signals would be superfluous. However, olfactometry tests do not allow us to exclude the possibility that in the field, these compounds may function as homing signals (see references in Hölldobler et al., 2004).

In this study, we demonstrated that *P. vermiculatus*, a solitary foraging harvester ant, produces and perceives compounds that function as trail pheromones in congeneric species. Given the ancestral nature of *P. vermiculatus*, this suggests that the character "production of semiochemicals" was fixed early in the evolutionary history of the group. If *P. vermiculatus* is able to perceive chemicals that in other *Pogonomyrmex* species are involved in group foraging behavior, then why does not this species (or, in general, the species of South America) exhibit group foraging? Several explanations are possible. First, it is probably not a question of the amount of compounds deposited by foragers because the total content in the glands of *P. vermiculatus* was similar to that of group foraging *Pogonomyrmex* species, and the content of one poison gland was shown to affect the locomotion patterns of test ants. Second, the absence of group foraging may be related to the number of foraging individuals. If the number of foragers is low, then, their efficiency in distributing the trail pheromones in the substratum would also be low, thus, constituting a limitation to the amplification of the signal (e.g., Beekman et al., 2001). In support of this proposal, the nests of the South American *Pogonomyrmex* species have fewer workers than the nests of North American species (Taber, 1998), and therefore, fewer individuals are assigned to foraging tasks (H. Torres-Contreras, personal observation). Third, because the number of ant species that forage and compete for seeds in arid habitats of Northern Chile is low (Medel, 1995) in comparison with the number of species present in the deserts of North America (Johnson, 2001, and references therein), group foraging may not be necessary in the South American species. Fourth, the proportion at which the three pyrazines were found in *P. vermiculatus* was similar to that found in *Pogonomyrmex maricopa*, a species documented as a solitary forager but which when confronted with a high abundance of resources exhibits recruitment of individuals and group foraging (Johnson, 2001). Thus, the pyrazines present in the poison gland of *P. vermiculatus* could play a role as a trail

pheromone and allow nestmate recruitment when there is a high availability and a patchy distribution of seeds in the habitat. The testing of these hypotheses is currently underway.

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