Adnexal Glands Chemistry of *Messor ebeninus* Forel (Formicidae: Myrmicinae)

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Anabasine is the major volatile product in the poison gland exudate of *Messor ebeninus*, acting as a defensive compound. Exudates of the poison gland also contain minor, yet unidentified, components that are possibly responsible for the alarm behavior that is also elicited by the venom.

Dufour's gland secretion is characterized by aliphatic hydrocarbons of which 1-pentadecene predominates. Upon exposure to Dufour's gland secretion the ants recruited to the emitting source, but did not exhibit any aggressive behavior. The possible concordant effects of both adnexal glands secretions is discussed.

Introduction

Among the abdominal glands of ants the two adnexal glands, Dufour's and the poison glands, are prominent. Although the poison gland secretion in the aculeate Hymenoptera served originally as defensive weapons, in some ant species this function has been abandoned in favor of a communicative role. The myrmicine ants are a good example of this evolutionary trend. Many species such as *Pogonomyrmex* spp. produce large amounts of proteinaceous venom which has a strictly defensive role [1]. Species of other genera, on the other hand, produce little proteinaceous material in their poison gland but instead manufacture an array of alkaloids which appear to have divers functions. For example, the venom of *Solenopsis* species are rich in 2,6-dialkylpiperidines [2, 3], or 3,5-dialkylpyrrolidines and pyrrolines [4, 5] which are effective toxins and are probably used for defense. In contrast, the venom of *Monomorium pharaonis* contains the alkaloid 3-butyl-5-methylindolizidine an active component in the trail following behaviour of this ant [6]. Likewise, methyl 4-methylpyrrole-2-carboxylate, a poison gland alkaloid in *Atta texana* function as the trail pheromone [7, 8]. Another poison gland alkaloid, anabaseine (3,4,5,6-tetrahydro-2,3′-bipyridine), found in two *Aphaenogaster* species, *A. fulva* and *A. tennessensis,* elicit alarm behavior in workers and apparently is a part of their alarm defense system [9].

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Field observations with *Messor ebeninus*, the subject of this study, revealed that it has a well-developed alarm behaviour as well as long food trails. It was therefore relevant to investigate the chemistry of the poison gland and the adjacent Dufour's gland secretions to determine the role of their constituents in these behaviors.

Materials and Methods

Colonies of *M. ebeninus* were collected near Tel Aviv and transferred in the laboratory to artificial nests placed on a foraging platforms (0.8×3 meters). The ants were regularly fed on a diet of honey and seeds, supplemented occasionally with dead insects. After a few days of acclimation the ants started foraging normally retrieving the food from the end of the foraging platform.

Extract of Dufour's and poison glands were prepared by dissecting the ants under chilled water and transferring the glands to vials containing pentan. The extracts were chemically analyzed on an LKB 9000 gas chromatograph-mass spectrometer fitted with 1.8 m 1% SP-1000 column programmed from 60–200 °C at 10 °C/min or a 1.8 m 1% OV-17 column programmed from 60–300 °C at 10 °C/min. The compounds were identified by the mass spectra as compared with the spectra of authentic compounds. The presence of each compounds was further ascertained by coinjection of standards and extracts. Amount of anabasine in the poison glands were determined by capillary gas chromatography using the internal standard method.

The alarm behaviour of the ants was observed qualitatively both in the field and in the laboratory. In the laboratory assays, the tested material was impregnated on a small piece of filter paper and placed at random on the foraging platform. The tested material included poison gland exudates, synthetic anabasine (racemic mixture, purchased from Sigma) and Dufour's gland extracts or its hydrocarbon constituents. In all cases a filter paper impregnated with pentan served as a control.

Recruitment of the ants toward the marked spot and the exhibition of open mandibles were recorded as a positive alarm response, while aggregation around the filter paper without any overt aggression was recorded simply as recruitment. Similar tests were conducted also in the field by placing the tested material in the proximity of the nest entrance.

Results and Discussion

Only one major compound was detected by GC–MS in the excised poison gland extracts. It eluted at 155 °C on the SP-1000 column and at 138 °C on OV-17. Its mass spectrum exhibited a molecular ion at *m/z* 162 (35%) and major fragments ions at *m/z* 84 (100%), 105 (37%), 106 (30%), 119 (25%), 120 (14%), 133 (32%) and 161 (18%). It was identified as anabasine (3-(2-piperidinyl)pyridine) by comparison of mass spectra and gas chromatographic retention time of the unknown and of an authentic sample of anabasine, and also by coinjection on the two columns. The absolute configuration of the unknown was not determined.

Although anabasine constitutes over 90% of the volatiles present in the secretion, it represent only 1% of the fluid present in the poison gland. The amount of anabasine in each gland, estimated by gas chromatography, was about 3.6 μg while the volume of the secretion as assessed by glandular measurements was around 0.35 μl. The amount of anabasine in virgin queens was only 50% of that of the workers and it dropped to 10% in 48 h postmated queens. GC–MS examination of Dufour's gland secretion revealed that it consisted of a series of n-alkanes and n-alkenes including 1-tridecene, tridecane, 1-pentadecene, the main component, pentadecane and traces of 1-heptadecene and heptadecane.

Workers of *M. ebeninus* reacted with typical alarm when exposed to crushed conspecific ants. They ran with open mandibles and outstretched antennae directly to the source of disturbance. Around the source the ants became very aggressive, biting every object while curling the abdomen toward it. In many of these instances a white secretion could be seen at the tip of the abdomen. This alarm was of short duration and faded completely within a few minutes although the ants remained in the vicinity of the filter paper for several additional minutes. Of all the body parts only crushed abdomen had the same effect on the ants, thus the ability of the adnexal glands secretion to elicit the same behaviour was tested.

Crushed poison glands indeed elicited alarm and aggressiveness in the ants which was of short duration. In contrast anabasine, its major volatile component, was totally devoided of activity. In a more refined analysis of the glandular exudate by capillary gas chromatography we noticed the presence of some minor components that were more volatile than anabasine. It is possible that these are the bioactive components, but their quantity was too small for proper identification. The response of the ants to Dufour's gland secretion or its hydrocarbon constituents was rather different. The ants upon perceiving the odour were attracted to the source but did not show any signs of alarm or aggression. This reaction was also of longer duration than the response to the poison gland secretion. The same results were obtained in the field when crushed glands were put near the nest entrances. Nests of *M. ebeninus* are rather large with multiple entrances, and placing crushed poison glands in the vicinity of nearby entrances not only excited the ants in the immediate vicinity but caused many more ants to come out of the nest entrances with overt aggression. The reaction to Dufour's gland secretion was milder causing only the ants in the nearest vicinity to recruit to the spot of emission.

The identification of anabasine in the poison gland of *M. ebeninus* constitutes the first exocrine product that has been reported from this genus. It also adds to the array of alkaloids produced by this gland in myrmicine ants. The finding of anabasine in the genus *Messor* is also of chemotaxonomic significance since the related unsaturated alkaloid anabaseine was found in *Aphaenogaster* species [9] and *Messor* was once considered as a subgenus of *Aphaenogaster* [10]. Although that is the first time that anabasine has been reported in insects, it occurs in a number of plants. It is a major alkaloid of *Anabasis aphylla* L. (Chenopodiaceae) and *Nicotinia glauca* Graham

(Solanaceae) [11]. Perhaps, because of its insecticidal properties, anabasine offers the plants protection from insect herbivors. Apparently this is also its function in the harvester ants as it is emitted from the poison gland when the ants are molested but apparently does not elicit any alarm. It is possible that it is a gustatory repellent rendering the ants unpalatable to some predators. A further indication that anabasine is a defensive compound stems from its reduced amounts in queens, especially after they have mated. Queens of this species are claustral nest founders and thus are less exposed to predation once they have dug their initial nest burrows.

In contrast to the alarm-defensive role of the poison gland secretion, the function of the secretion emanating from Dufour's gland is more elusive. Although our bioassays indicate that the secretion causes recruitment of the ants into the emission source, there was not any overt aggression involved in this behavior which preclude a strict alarm function. It is possible that the glandular secretion plays a role in the recruitment to a food source as found for *M. rufitarsis* [12, 13]. We, however, were unable to induce trail following on our foraging platform using Dufour's gland secretion alone. Full trail following in this species thence may involve secretions of more than one abdominal gland. In many cases when the ants were alarmed a frosty secretion could be seen at the tip of the abdomen suggesting that the secretion of the two adnexal glands may have been emitted together. This frosty appearance of the secretion is due to the fact that the poison gland secretion is water soluble while that of Dufour's gland is highly lipophilic. In this case there is seemingly a sort of synergism in the function of the two secretions. The poison gland secretion act immediately in defense and alarm while the longer recruiting effect of Dufour's gland secretion insure that the ants stay in the vicinity of the potential danger in case of further need. A similar synergism was found also in *Camponotus* [14]. This latter function does not preclude the possibility that Dufour's gland secretion plays a role in mass recruitment of the ants toward a food source. It just demonstrates another example of the parsimony in the pheromonal systems of ants.

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