

Mandibular Gland Secretions as Alarm Pheromones in Two Species of the Desert Ant *Cataglyphis*

Abraham Hefetz

Department of Zoology, George S. Wise Faculty of Life Sciences, Tel Aviv University, Ramat Aviv 69978, Israel

Helen A. Lloyd

Laboratory of Chemistry, National Heart Lung and Blood Institute, Bethesda MD 20205, U.S.A.

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Mandibular gland secretion of two *Cataglyphis* species were chemically analyzed by combined gas chromatography mass spectrometry. The exudates produced by *C. bombycina* consisted of citronellol and geraniol while that of *C. nigra* contained only geraniol.

The reaction of both species to the glandular secretions was alarm and recruitment. While the reaction of *C. bombycina* was strong, that of *C. nigra* was relatively mild.

Introduction

The alarm defence system of ants relies mostly on the products of three exocrine glands: mandibular, Dufour's and poison glands. In some cases the ants species use all three of them while in other species only one or two glands are utilized for this purpose. In *Acanthomyops claviger* for example the secretions of all three glands are reported to elicit alarm response in workers [1]. In *Polyrhachis simplex*, on the other hand, the mandibular gland products enhance the alertness of the ants while the secretions emitted from Dufour's and poison glands induce alarm and recruitment [2]. In the Formicinae the use of formic acid for defence is ubiquitous, and in many species this volatile acid had become also the alarm pheromone [3]. Indeed in many formicine ants the mandibular glands are reduced and are apparently non functional. Some species however had retained large mandibular glands, among which are two *Cataglyphis* species that are the subject of this report.

Materials and Methods

Two species of *Cataglyphis* were investigated: *C. nigra* collected from Tel Aviv, and *C. bombycina* collected from Eilat, Israel. The ants colonies were brought to the laboratory where they were reared on foraging tables (3 m × 3 m).

In order to investigate the chemical nature of the glandular secretions, mandibular glands were excised from chilled ants and placed in methylene chloride for extraction. Alternatively, whole heads were removed directly into a vial containing methylene chloride. Chemical analysis was performed by combined gas chromatography mass spectrometry (LKB 9000). Separation of the glandular components was achieved utilizing a 10% SP 1000 column, temperature programmed from 40 °C to 250 °C at 10 °C per minute. The eluting compounds were identified from their respective mass fragmentation patterns as compared to those of authentic compounds. Further confirmation of the identity of the various compounds was achieved by coinjecting the glandular exudates and the synthetic compounds on two capillary columns: At 1000 (polar) and SE 30 (non polar).

Behavioral studies were conducted in the laboratory for both species and in the field for *C. nigra*, using either crushed mandibular glands or synthetic compounds. In the laboratory assays, the tested material was impregnated on a small piece of filter paper and placed at random on the foraging table. Recruitment of the ants towards the marked spot and the exhibition of open mandibles were recorded as a positive response. Similar tests were conducted also in the field by placing the tested material in the proximity of the nest entrance.

Results and Discussion

Crushed mandibular glands of the two species investigated emitted a fragrance characteristic of ter-

Reprint requests to Dr. A. Hefetz.

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penoid compounds. Indeed the chemical analysis of the glandular exudates revealed the presence of two such compounds in *C. bombycina* but only one in *C. nigra*. The first compound eluted at 170 °C; it had a molecular weight of 156 and its mass spectrum was identical with that of synthetic citronellol. The natural compound also coeluted with citronellol on the two GC columns. Citronellol occurred only in *C. bombycina*, comprising approximately 50% of the secretion. The second compound eluted at 180 °C and was common to both species. This compound had a molecular weight of 154 and its spectrum was identical with that of geraniol. The identity of the second natural compound as geraniol was also corroborated by coinjections with an authentic sample. Although quantitative analyses were not done, the larger mandibular glands of *C. bombycina* had a more abundant secretion than those of *C. nigra*.

The response of the two species to their mandibular gland secretion or alternatively to the synthetic compounds was also different. In the laboratory assays *C. bombycina* reacted very strongly to crushed mandibular glands. The response included fast running towards the emitting source with open mandibles, and apparent aggression once the ants reached the source. The experiments conducted using geraniol and citronellol, separately or combined, resulted in the same stereotyped behavior. In contrast to the strong reaction exhibited by *C. bombycina*, *C. nigra* responded very mildly to either crushed mandibular glands or synthetic geraniol. In the laboratory, only few ants recruited near the emitting source with no overt aggression. In the field, when the extracts were applied near the nest entrance, it elicited an alarm response in the ants that were guarding the nest. In this respect synthetic geraniol had as much activity as crushed glands. Interestingly citronellol, although not present in the glands of *C. nigra* was as effective as geraniol.

The differences in the intensity of the response of the species investigated to crushed mandibular glands correlates with their observed aggressiveness in the field. *C. bombycina* is very aggressive attacking fiercely every moving insect in its foraging area with its enlarged and sharpened mandibles. *C. nigra* on the other hand, has smaller mandibles and is more docile and attacks only when it is disturbed. These differences in alarm response were also observed when the ants were exposed to formic acid, their poison gland product [4].

The occurrence of citronellol or geraniol in other species of ants is rare. Geraniol is known to occur only in one myrmecine species *Atta sexdens* as a product of the mandibular glands [5] where, as in *Cataglyphis* it serves as an alarm pheromone. Citronellol is the alarm pheromone of two other *Atta* species: *A. capiguara* and *A. laevigata* [5] and of the females of the formicine species *Lasius alienus* [6] and *L. umbratus* [7]. In *L. umbratus*, citronellol constitutes the major product of the secretion and is considered a defensive compound while citronellal is the effective alarm substance. Citronellol also occurs in the male mandibular glands of *L. neoniger* and *Acanthomyops claviger* but its function there is unknown [8].

It is interesting to note that the formicine ants in which these terpenoid compounds occur, use mostly their poison and Dufour's gland secretions for alarm and defence. This is also the case in the *Cataglyphis* species investigated. Formic acid is by far more effective as an alarm elicitor than the mandibular gland products [4] and it is puzzling that there should be a dual alarm system in these ants. It is possible that mandibular gland secretions were used first as alarm pheromones but that later, as the more volatile formic acid was adopted as a defensive substance, this compound evolved adaptively also as an alarm pheromone.

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