Dufour's Gland Composition in the Desert Ant *Cataglyphis:* Species Specificity and Population Differences

Abraham Hefetz

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

Alain Lenoir

Laboratoire d'Ethologie et Sociobiologie, Universite Paris Nord, Av. J. B. Clement, F-93430 Villentaneuse, France

Z. Naturforsch. **47c**, 285–289 (1992); received May 7/December 30, 1991

Cataglyphis, Dufour's Gland, Hydrocarbons, Species Specificity, Geographic Variations

Dufour's gland secretion of several species of the desert ant *Cataglyphis* from different geographical localities was analyzed. The secretions constituted mostly of alkanes ranging from undecane to nonadecane. Species specificity is expressed as variations in the major component as well as the relative intensities of the additional constituents. Phylogenetically related species that are allopatric exhibited similar secretory composition whereas their sympatric counterparts had disparate composition, suggesting that character displacement occurred. Analyses of colonies of *C. cursor* from different localities also showed divergence in their glandular composition.

Introduction

Dufour's gland secretion in formicine ants was reported to function, in general, as a part of their alarm-defense system, often complementing the action of formic acid [1, 2]. The response of the ants to pure Dufour's gland secretion, however, differed between the species. In *Acanthomyops claviger,* for example, it elicited strong alarm and aggression [3], while in *Camponotus sericeus* or *Cataglyphis niger* the reaction to the secretion was general recruitment without any overt aggression [4]. A comparative study of 12 species of *Camponotus,* exhibiting different foraging ecology, led to the suggestion that it is in species that employ mass foraging that Dufour's gland secretion elicit strong alarm. On the other hand, in species that forage singly or in tandem the secretions have at most a recruiting effect [4].

Chemically, Dufour's gland secretions of most formicine species have a complex composition, albeit with a simple chemistry. There are species, like in the genus *Cataglyphis,* in which the secretion contained mostly aliphatic hydrocarbons [4–6]. Some species of *Camponotus* produced the same array of hydrocarbons as in *Cataglyphis,* while in other the secretions were dominated by oxygenated compounds [7].

In a previous study [4], Dufour's gland chemistry of several species of *Cataglyphis* occurring in Israel was studied, demonstrating that the composition was species specific. We present here results of the chemical analyses of 4 additional species from remote localities of the genus distribution. *Cataglyphis viatica* and *C. bicolor* collected from North Africa, and *C. iberica* and *C. cursor,* from southern Europe. We also present analyses of different populations of *C. cursor.* These comparative studies enabled us to hypothesize on the evolutionary significance of the chemical diversity of the glandular secretions.

Materials and Methods

The various species of *Cataglyphis* were collected as follows: *C. niger* from Tel Aviv, Israel; *C. viatica* and *C. bicolor* from Tunisia; *C. iberica* from northern Spain and *C. cursor,* from different populations in Spain and the south of France.

Dufour's gland were removed from dissected ants and placed immediately in pentane for extraction. Alternatively, whole abdomens were extracted. Qualitative chemical analyses were performed by combined gas chromatography and mass spectrometry, and the identity of the compounds confirmed by coinjection with synthetic standards.

Reprint requests to Prof. A. Hefetz.

Verlag der Zeitschrift für Naturforschung, D-W-7400 Tübingen
0939–5075/92/0300–0285 $ 01.30/0

Quantitative analyses were performed by capillary gas chromatography using a 30 m SE-30 column that was temperature programmed from 60–250 °C at 8 °C with a 5 min hold at the initial temperature. The analyses of the secretions of *C. niger*, *C. viatica*, and *C. bicolor* were done using individual glandular exudates. While that of *C. iberica* and the various populations of *C. cursor* were done using pooled samples of 10 glands. All samples were analyzed at least twice at a random order. The degree of similarity between the secretionary compositions of the various species or populations of *C. cursor* was estimated by a cluster analysis of cases [8], and its significance was tested by a Wilcoxon test [9].

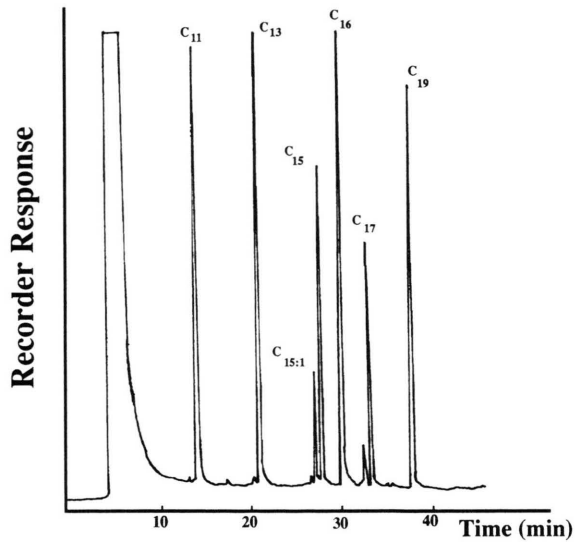

Fig. 1. Gas chromatogram of Dufour's gland secretion of *Cataglyphis cursor* from the St. Hyppolite population. Dissected glands were pooled from 10 ants and extracted in pentane. The sample was analyzed by gas chromatography using a 30 m SE-30 capillary column that was temperature programmed from 60–250 °C at 8 °C with a 5 min hold at the initial temperature.

Results and Discussion

Chemical composition

Dufour's gland secretion in the species of *Cataglyphis* studied is composed of a series of low boiling saturated hydrocarbons ranging from undecane and nonadecane (Fig. 1). Analyses of the concentrated secretions often revealed the presence of minor amounts of the corresponding alkenes, as well as trace amounts of additional, unidentified, oxygenated components. The composition of the secretion produced by Dufour's gland in the species studied here was not qualitatively different from the secretion of other species of *Cataglyphis* [4–6], and may be stated as characteristics to the genus.

Species specificity

The comparative analysis of the secretions of the various species studied was limited to the abundant components, *i.e.*, the alkanes, emphasizing their relative occurrence in the species investigated. The main differences were expressed in the identity of the major components and the relative intensities of all components present in the secretion (Table I).

Table I. Dufour's gland chemical compositions (alkanes only) of *Cataglyphis* species. The data for *C. nodus* are taken from Hefetz and Orion 1982 [4]. The numbers within the table indicate the relative intensity (expressed as the percentage of the total amount of secretion as inferred from the peak areas) of each of the components as revealed by quantitative gas chromatography. Chromatography conditions were as depicted in Fig. 1.

	C. niger	*C. cursor*	*C. iberica*	*C. viatica*	*C. bicolor*	*C. nodus*
Undecane	12	7	6	18	8	6
Dodecane	2	2	0	1	0	1
Tridecane	45	66	15	80	49	15
Tetradecane	3	2	2	0	2	2
Pentadecane	37	8	75	1	39	68
Hexadecane	0	8	1	0	0	0
Heptadecane	1	2	1	0	2	7
Octadecane	0	0	0	0	0	0
Nonadecane	0	5	0	0	0	0

The identity of the major components is characteristics to the species, whereas the relative amounts of the accompanying components further emphasize species specificity. For instance, the secretion of *C. iberica* contained mostly pentadecane in contrast to that of *C. viatica* that contained mostly tridecane. The secretion of *C. niger* and that of *C. bicolor* were almost identical having both tridecane and pentadecane as major components, with low amounts of undecane and traces of dodecane and heptadecane. The secretion of *C. cursor* was the most complex including the whole homologous series from undecane to nonadecane with tridecane as a major component (Fig. 1). According to the results of this study species specificity can be obtained on the basis of 4 components only, out of the 9 compounds that may be present in any secretion. This is in accordance with the prediction of the number of components needed for species specificity that was estimated using data obtained from halictine bees and that were also based on Dufour's gland composition [10].

Geographic distribution and species specificity

The degree of similarity in the secretionary composition between the species, based on the amalgamation distances before clustering, is presented in Table II. As mentioned above the secretory compositions of *C. bicolor* and *C. niger* were alike. This was further verified by a cluster analysis of cases using data obtained from individual analyses of members of these species. When the degree of similarity between individuals *C. bicolor* and individuals of *C. niger* was compared to the degree of similarity within individuals of *C. niger,* there was a slight difference (P = 0.002). When the opposite comparison was done, *e.g.*, the degree of similarity between individuals *C. bicolor* and *C. niger* was compared to the similarity within individuals of *C. bicolor* it was not significant (P = 0.5). This asymmetry was caused by the higher variability of individuals of *C. bicolor,* and indicates that if there is a difference between the species it is only a slight one.

The similarity between these two species is not surprising since both belong to the same species group, and were considered as subspecies [11]. The interesting point is that another member of this species group *C. nodus* is significantly different from *C. niger* [4] (Fig. 2). The explanation for these more pronounced differences between *C. niger* and *C. nodus* may be due to the fact that they are at least in part of their distribution sympatric. The population of *C. bicolor* examined, however, is totally isolated from the former two species, and is limited to the deserts of North Africa. It is possible that the differences between

Table II. Similarity between the compositions of Dufour's gland secretions of various *Cataglyphis* species. The samples were analyzed by cluster analysis of cases according to Dixon 1968 [8], based on the relative intensities of the various secretory components. Samples from each species were chromatographed at least twice, and each sample constituted a case for the clustering. The degree of similarity between the cases is expressed as an amalgamation distance. For statistical testing of the significance of species specificity, the median amalgamation distances between the species before clustering were used. Numbers in parenthesis indicate the degree of significance assessed by a Wilcoxon test.

Species	*C. niger*	*C. cursor*	*C. iberica*	*C. viatica*	*C. bicolor*
C. niger	15.5	*	*	*	*
C.cursor	79.1 (p = 0.000)	33.1	*	*	*
C. iberica	85.1 (p = 0.000)	145.6 (p = 0.000)	15.3	*	*
C. viatica	81.5 (p = 0.000)	45.2 (p = 0.05)	164.1 (p = 0.000)	14.1	*
C. bicolor	20.6 (p = 0.002)	74.4 (p = 0.000)	83.1 (p = 0.000)	81.4 (p = 0.000)	21.7

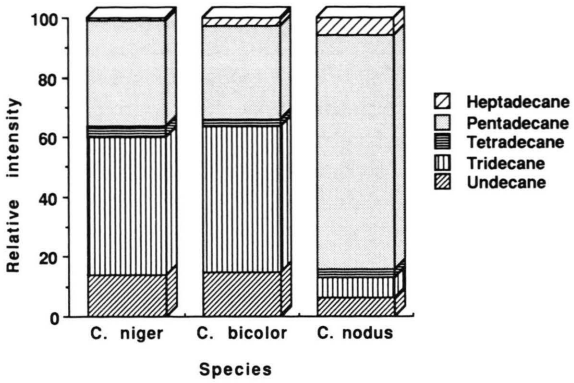

Fig. 2. Composition of the alkanes fraction of Dufour's gland secretion of three species of *Cataglyphis* belonging to the "bicolor group". The data for *C. nodus* are taken from Hefetz and Orion 1982 [4]. For chromatographic condition and quantification of the secretory composition see Materials and Methods, Fig. 1, and Table.

C. nodus and *C. niger* reflect a competitive selection on the signal emanating from Dufour's gland. Laboratory assays with Dufour's gland secretion indicated its role as a general recruiting agent, but not as an alarm agent [4]. If in nature the secretion serves as a general home range marker, it is conceivable that selection pressures on sympatric species using similar secretions resulted in changes in the proportions of the secretion components. *C. bicolor* being taxonomically related to these species, but in the absence of their competition, conserved the old composition of this species group. It would be interesting to verify whether other species of the "bicolor group" which are sympatric with *C. bicolor* show similar disparity in Dufour's secretionary composition. Unfortunately, we were not able to obtain Dufour's gland samples of such a species, and this point remains speculative at the moment.

The tendency of Dufour's gland secretion to diversify is well expressed when the secretions of *C. cursor* from different populations were compared using a cluster analysis of cases. Although, because of the small sample size, it was not possible to fully test the statistical significance of the differences exhibited, limited conclusions could still be drawn from such a comparison (Fig. 3). The population of *C. cursor* from Apt is indistinguishable from that of Le Muy, but is distinct from all other populations studied. Likewise, the populations of Montpellier and St. Hyppolite are distinct from all other population studied, as well as between them. Similar results were obtained when cuticular hydrocarbons were investigated [12]. High congruence between the populations of *C. cursor* originating from Le Muy and Apt was found, suggesting that these populations of *C. cursor* correspond to the typical *C. cursor* originally described by Fonscolombe in 1846 [13]. Other populations located at the west side of the Rhon are more heterogeneous and must correspond to *C. piliscapa* [14]. This distinction is discussed by Agosti and Collingwood 1987 [15].

Dufour's gland secretion in formicine ants was reported to function as an additive to formic acid, the poison gland product, that facilitates its penetration through the hydrophobic cuticle. It was also reported as an alarm pheromone in many formicine species [1]. While it is possible that these two functions exist in different species, or may even act in cohort, the limited data on the diversity of the secretion between species and within species suggest that the information encoded within this secretion is more complex. In many of formicine

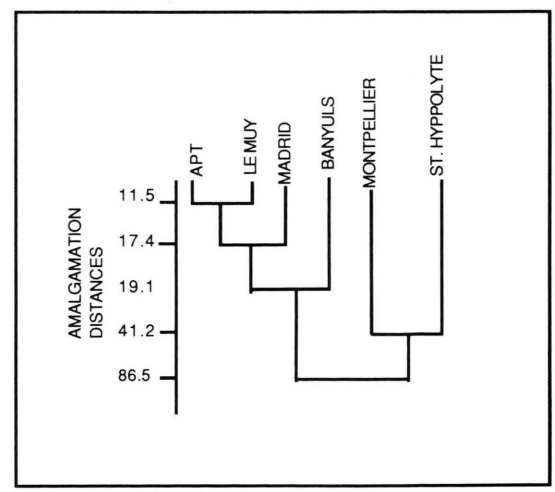

Fig. 3. Cluster analysis of cases, based on Dufour's gland secretion, of various populations of *C. cursor*. Pooled glands were analyzed by gas chromatography and the relative amounts of the various components were calculated from their peak areas. The data were subjected to a cluster analysis of cases according to Dixon 1968 [8]. Samples from each species were chromatographed at least twice, and each sample constituted a case for the clustering. The degree of similarity between the cases is expressed as an amalgamation distance.

species investigated, the glandular secretions cause general alert as well as recruitment, but little aggression. This is in opposition to the reaction towards formic acid which is almost always high excitement with frequent frenzied attacks at the source. We suggest here that Dufour's gland secretion act as a general marker of home range of the ant colony. Such markings may be characteristics of individually foraging species such as *Cataglyphis*. A specific signal as such also explain why should the secretion be sometimes so complex, and why high variability between species and, as in the case of *C. cursor* between population within a species, is high. This also explains why taxonomically related species that are sympatric have diverse secretions, while as taxonomically related, but allopatric species retained the similarities in the secretion.

[1] M. S. Blum and H. R. Hermann, in: Arthropod Venoms (S. Bettini, ed.), p. 801, Springer Verlag, New York 1978.
[2] B. Hölldobler and E. O. Wilson, The Ants, 732 pp., Harvard University Press, Cambridge, Mass. 1990.
[3] F. E. Regnier and E. O. Wilson, J. Insect Physiol. **14,** 955 (1968).
[4] A. Hefetz and T. Orion, Isr. J. Entomol. **16,** 87 (1982).
[5] M. F. Ali, J. P. J. Billen, B. D. Jackson, and E. D. Morgan, Biochem. Syst. Ecol. **16,** 647 (1988).
[6] E. D. Morgan, D. Agosti, and S. J. Keegans, in: Social Insects and their Environment, Proc. 11th Inter. Congr. IUSSI (G. K. Veeresh, B. Malik, and C. A. Viraktamath, eds.), p. 515 (1990).
[7] G. Bergström and J. Lofqvist, Entomol. Scand. **3,** 225 (1972).
[8] W. J. Dixon, BMDP – Statistical Software, 726 pp., University of California Press, Berkeley 1968.
[9] R. R. Sokal and P. J. Rohlf, Biometry, the Principles and Practice of Statistics in Biological Research, 859 pp., W. H. Freeman and Co., San Francisco 1969.
[10] A. Hefetz and D. Graur, Biochem. Syst. Ecol. **16,** 557 (1988).
[11] F. Santschi, Rev. Suisse Zool. **36,** 25 (1929).
[12] E. Nowbahari, A. Lenoir, J. L. Clement, C. Lange, A. G. Bagneres, and C. Joulie, Biochem. Syst. Ecol. **18,** 63 (1990).
[13] B. Fonscolombe, Ann. Soc. Ent. Fr. **4,** 39 (1846).
[14] A. Forel, Mitt. Nat. Hist. Hamburg **18,** 43 (1901).
[15] D. Agosti and C. A. Collingwood, Mitt. Schw. Ent. Ges. **60,** 261 (1987).