

USING CHEMICAL TOOLS TO DISCRIMINATE TAPINOMA SPECIES.

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Study context: This study is the latest step in our research on the Argentine ant (Linepithema humile) invasion along the Mediterranean coast (Blight et al. 2010a), with the objective of developing biological tools to contain this invasion. We previously showed that in the laboratory, T.nigerrimum workers were able to resist and even in some cases to exterminate the Argentine ant (Blight et al. 2010b). In order to contain a potential new settlement of the Argentine ant in the field, we propose erecting a "biological barrier" in the form of T. nigerrimum nest transplantations. These transplantations are likely to thrive given that T. nigerrimum is native to the zones in question. However, several species of Tapinoma, morphologically not easily distinguishable, are present along the Mediterranean coast and it is difficult to discriminate among Tapinoma species, the only variations being in the clypeal cleft shape of queen or workers. Currently, male genitalia analysis provides the only secure method of differentiation.

Study goal: In order to quickly discriminate Tapinoma species on the basis of only one worker, we used hydrocarbon (HC) profiles to provide a discrimination pattern for each species. Characterization of the HC of each profile completes this study.

Materials & Methods: Four species of Tapinoma (T. erraticum, T. israelis, T. madeirense and T. nigerrimum) were collected from Israel (T. israelis) and continental France (*T. erraticum:* Azay sur Cher, Indre & Loire; *T. madeirense:* Fréjus, Var and *T. nigerrimum:* Auriol, Var) and were morphologically identified.

We analysed HC profiles and identified HC by gas chromatography/mass spectrometry using an Agilent 6890N GC equipped with Chrompack CPSil5WCOT apolar capillary

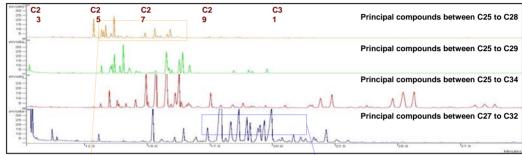
column (fused silica, 25m x 0.25 mm; thickness of stationary phase, 0.12µm) and injector in splitless mode. Initial oven temperature was held at 100°C for 1 min and then increased at a rate of 10°C/min to 220°C, then by 3°C/min to 320°C, where it was held constant for 15 min. Compounds were identified on the basis of their mass spectra and comparison with standard data, when available. Individual workers (5 per species) were immersed in 10µl of hexane for 15 min. 2µl extract were injected.

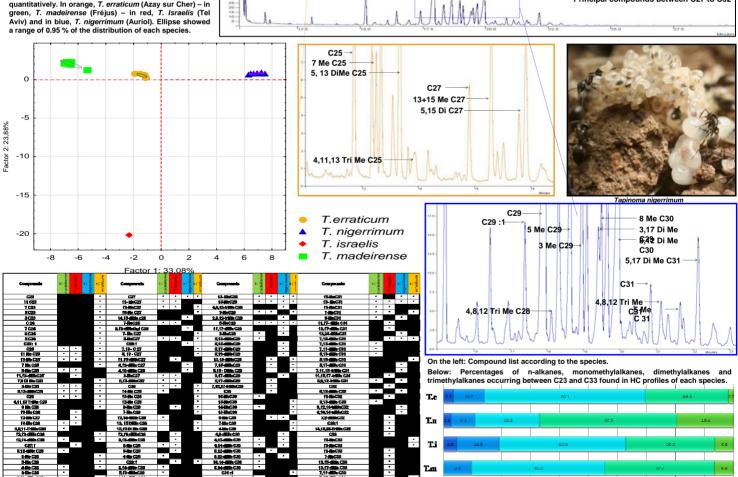
Between-species variability in profile composition was assessed using multivariate principal component analysis (PCA; Statistica v.6) based on the proportions of the 154 major cuticular hydrocarbons

Results:

Cuticular profiles of the four Tapinoma species. In orange, HC profile of *T. erraticum* (Azay sur Cher) – in green, HC profile of *T. madeirense* (Fréjus) – in red, HC profile of *T. Israelis* (Tel Aviv) and in blue, HC profile of *T. nigerrimum* (Auriol).

The PCA based on 154 major cuticular hydrocarbons of the four species, and 5 individuals per species revealed clear discrimination among these four species. Chemical profiles between the species differed qualitatively and quantitatively. In orange, *T. erraticum* (Azay sur Cher) – in green, *T. madeirense* (Fréjus) – in red, *T. Israelis* (Tel





Conclusion:

- Our results demonstrated a clear discrimination among species.
- There was a large diversity of specific dimethyl alkanes that makes them likely candidates for nestmate and species discrimination signals.
- We found 154 cuticular hydrocarbons occuring between C23 and C33 (n-alkanes, monomethyl alkanes, dimethyl alkanes and trimethyl alkanes).

Cuticular HC profiles are a quick discriminant tool for ecological studies.

Blight O., Renucci M., Tirard A., Orgeas J. and Provost E., 2010a. A new colony structure of the invas Blight O., Provost E., Renucci M., Tirard A. & Orgeas J. (2010b) A native ant armed to limit the spread ive Argentine ant (*Linepithema humile*) in Southern Europe. *Biological Invasions* 12:1491–1497 of the Arnentine ant. *Biological Invasions* . Published in line DOI 10.1007/s10530-010-9770-3













■C=

■ N alkane

■Me alkane

DiMe alkane



TriMe alkane

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Communication among social insects is fundamental between individuals, castes and societies. This is achieved through chemical recognition and ensures that altruistic acts are directed towards relatives. The nestmate and species discrimination in ants is based on antennal detection of non-volatile chemicals found on the cuticle. These cuticular compounds are species specific and can be used as biosystematic tools. Actually, discriminating some of the *Tapinoma* species is a difficult undertaking with only variations in the clypeal cleft shape of queen or workers. Currently male genitalia analysis provides the only secure method for differentiation. In order to simplify the differentiation of five species of *Tapinoma* (*T. erraticum*, *T. Israelis*, *T. madeirense*, *T. nigerrimum*, and *T. simrothi*) we used gas chromatography coupled with GC-mass spectrometry to identify, compare and quantify cuticular hydrocarbons (CHCs). We found that each species possess unique pattern of CHCs. Across the five species, 154 CHCs were identified. We found n-alkanes, monomethylalkanes, dimethylalkanes and trimethylalkanes occurring between C23 and C33. Unlike the n-alkanes and monomethylalkanes, there was a large diversity of specific dimethylalkanes that makes them likely candidates for nestmate and species discrimination signals.

Keywords: *Tapinoma*, Nestmate recognition, cuticular hydrocarbons, gas chromatography, GC-mass spectrometry, biosystematic.

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