Differentiation of the ant genus Tapinoma from the Mediterranean Basin by species-specific cuticular hydrocarbon profiles

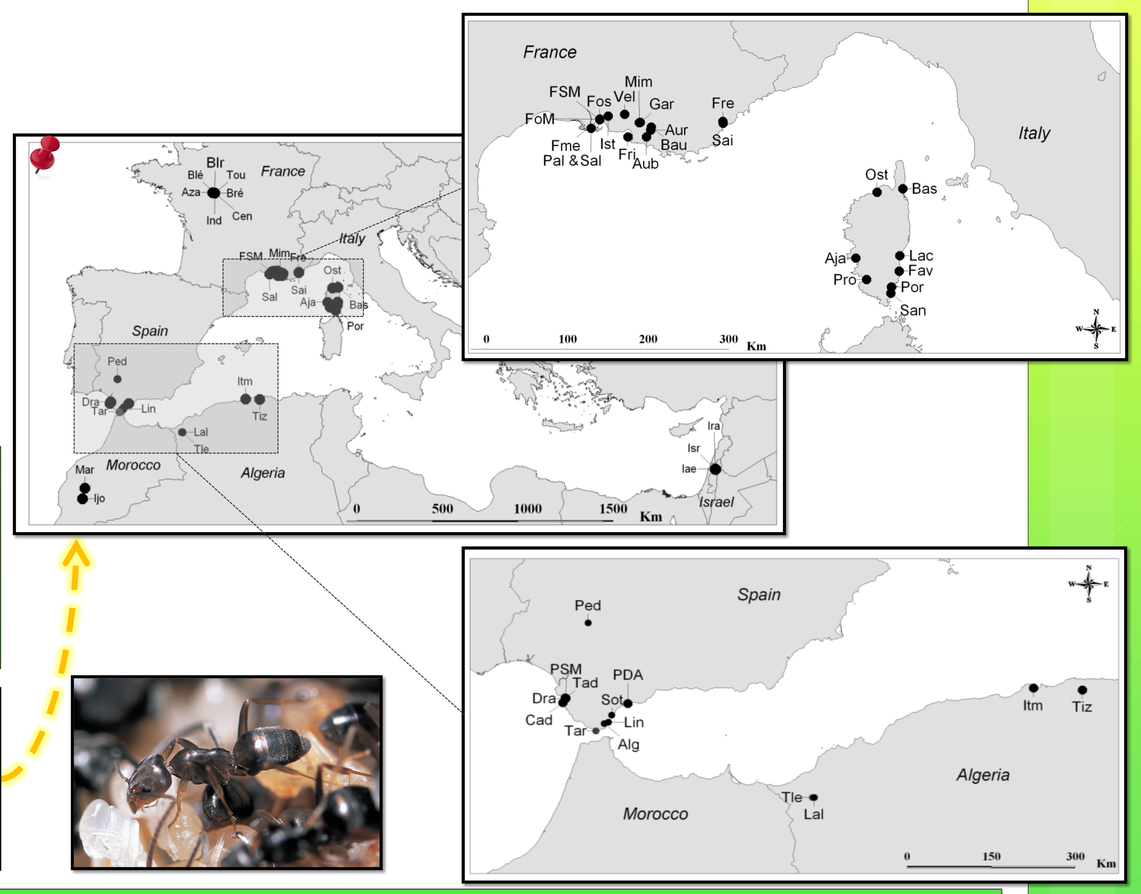
Berville L[1]*., Hefetz A[2]., Lenoir A[3]., Renucci M[1]., Blight O[1]., & E. Provost[1]

[1] Aix-Marseille Université, CNRS, IMBE, 7263 UMR, Campus Aix Technopôle Arbois-Méditerranée, Pavillon Villemin, F-13545 Aix-en-Provence Cedex 04, France. [2] Department of Zoology, George S. Wise Faculty of Life Sciences, Tel Aviv University, Ramat Aviv, 69978, Israel. [3] IRBI, UMR CNRS 7261, Université François Rabelais, Faculté des Sciences et Techniques, Parc Grandmont, 37200 Tours, France

* *laurence.berville@imbe.fr or laurence-berville@hotmail.fr*

Study context

Correct species identification is a precondition for biological study; yet despite a long history of morphological investigations, the systematic position of many ant species remains unclear. Like any phenotypic character, cuticular hydrocarbons (CHCs) are reliable indicators of species identity. Here, we compared and identified cuticular hydrocarbon profiles of workers of several species of *Tapinoma.*



Materials & Methods

50 Colony fragments were collected in **Algeria, France** (Corsica and continental France),**Morocco, Israel** and **Spain**. The nesting sites sampled ranged over **1900 km** from the northern to the southern part and over **4800 km** from the eastern to the western part of the study area.

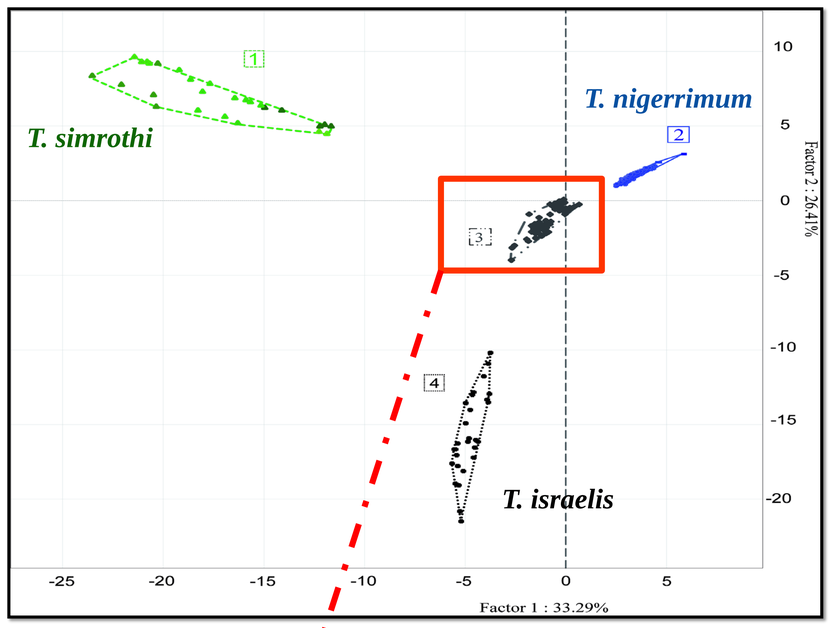
7 workers from each nesting site (**n =350**) were immersed separately in 5µl of hexane (15 min) in order to extract and estimate cuticular hydrocarbons. 3µl of this extract was used for capillary gas chromatography (**GC**), carried out using a Varian 3 900 gas chromatograph equipped with a flame ionization detector (FID) and a Chrompack CPSil5WCOT apolar capillary column and interfaced with Star 5.5 (Varian) software. Oven temperature was held at 100°C, then increased to 220°C at 10°C/min, then to 320°C at 3°C/min and finally held at constant temperature for 10 min. The injector and flame-ionization detector were at 280 and 250 °C respectively. Quantitative data were obtained by integrating peaks.

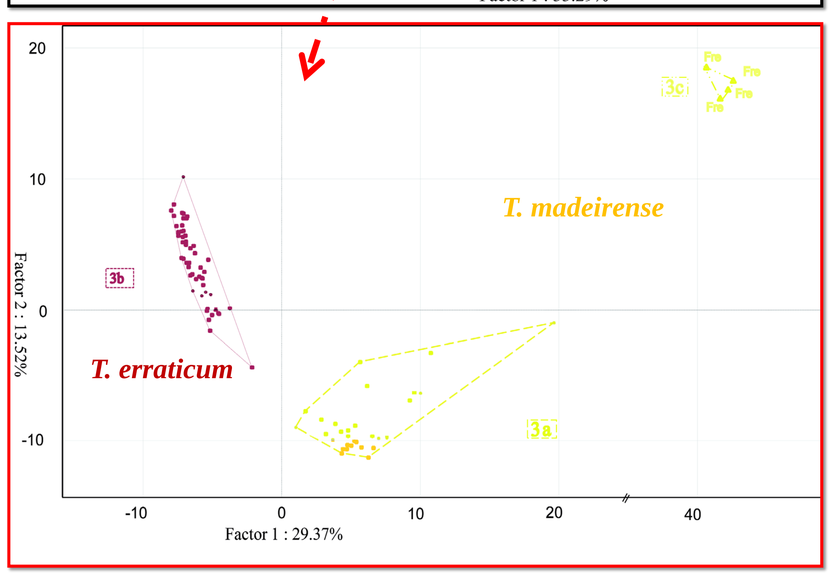
Total body washes of 20 pooled workers from each species were immersed in 100µl of hexane (20 min) and used for extraction. 4µl of each extract were run into an Agilent 6890N GC equipped with Chrompack CPSil5WCOT apolar capillary column. The GC was coupled with a 5375 Agilent Technologies Mass Spectrometer (**GC-MS**). The carrier gas was helium at 1 ml/min with the injector in splitless mode. Oven temperature was isothermal at 70°C for 1 min, followed by 30°C/min to 180°C, then increased at a rate of 5°C/min to 320°C, and finally held for15 min.

Results :

T. nigerrimum

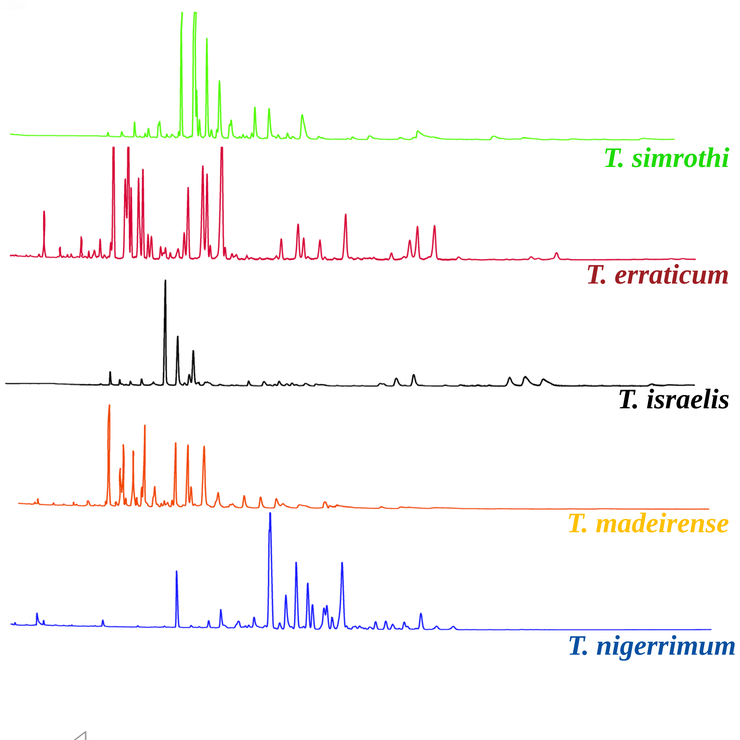
PCA based on the proportion of the 168 CHCs of worker from the 50 nesting sites. *K*-means cluster analysis distinguishes 4 groups.

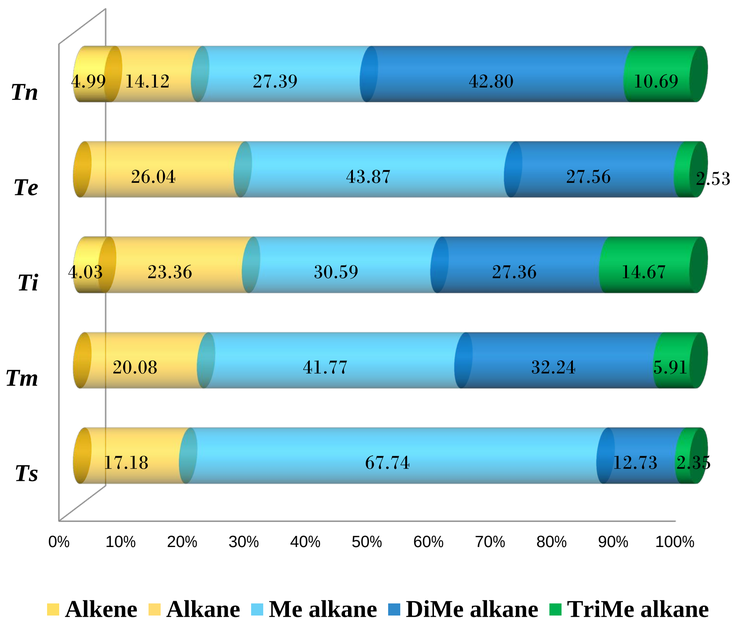




New PCA based on the proportion of the 77 CHCs of worker from the 3th group. *K*-means cluster analysis distinguishes 3 groups.

Cuticular profiles of the 5 *Tapinoma* species :





Quantitative presence in percent of the 5 distinct hydrocarbons groups according to the species.

T. s **: 70 CHCs => C25 to TriMe C37**

T. e **: 39 CHCs => C25 to DiMe C31**

T. is **: 65 CHCs => C25 to TriMeC35**

T. m **: 62 CHCs => C25 to DiMe C35**

T. N **: 35 CHCs => C27 to DiMe C32**

Conclusions :

- **The chemistry of the CHC bouquet is shown to be a good tool for taxonomists, being species-stable over thousands of kilometers.**
- **Extremely high diversity in the chemical recognition cues :**
  - Only 3CHCs in common to all species (C27, C29& 13 Me C31)
  - 108 CHCs (64.28%) present only in one species
  - Only *T. nigerrimum* and *T. israele* present *n*-alkenes
  - Across the 5 species, wide range of CHCs (168) were found, occuring between C25 to C 37

Max Planck Institute for Chemical Ecology

Acknowledgements:

We are very grateful to M. Boukhemza and N. Boukhemza – Zemmouri (University of Tizi-Ouzou), A. Payan, E. Berville, P. Ponel (IMBE) and Y. Ben Mordechai (Israel) for providing some field ants, and to Jean-Philippe Christidès (IRBI) for his help and advice with the GC. This work was supported by the Provence-Alpes-Côtes d'Azur Region.