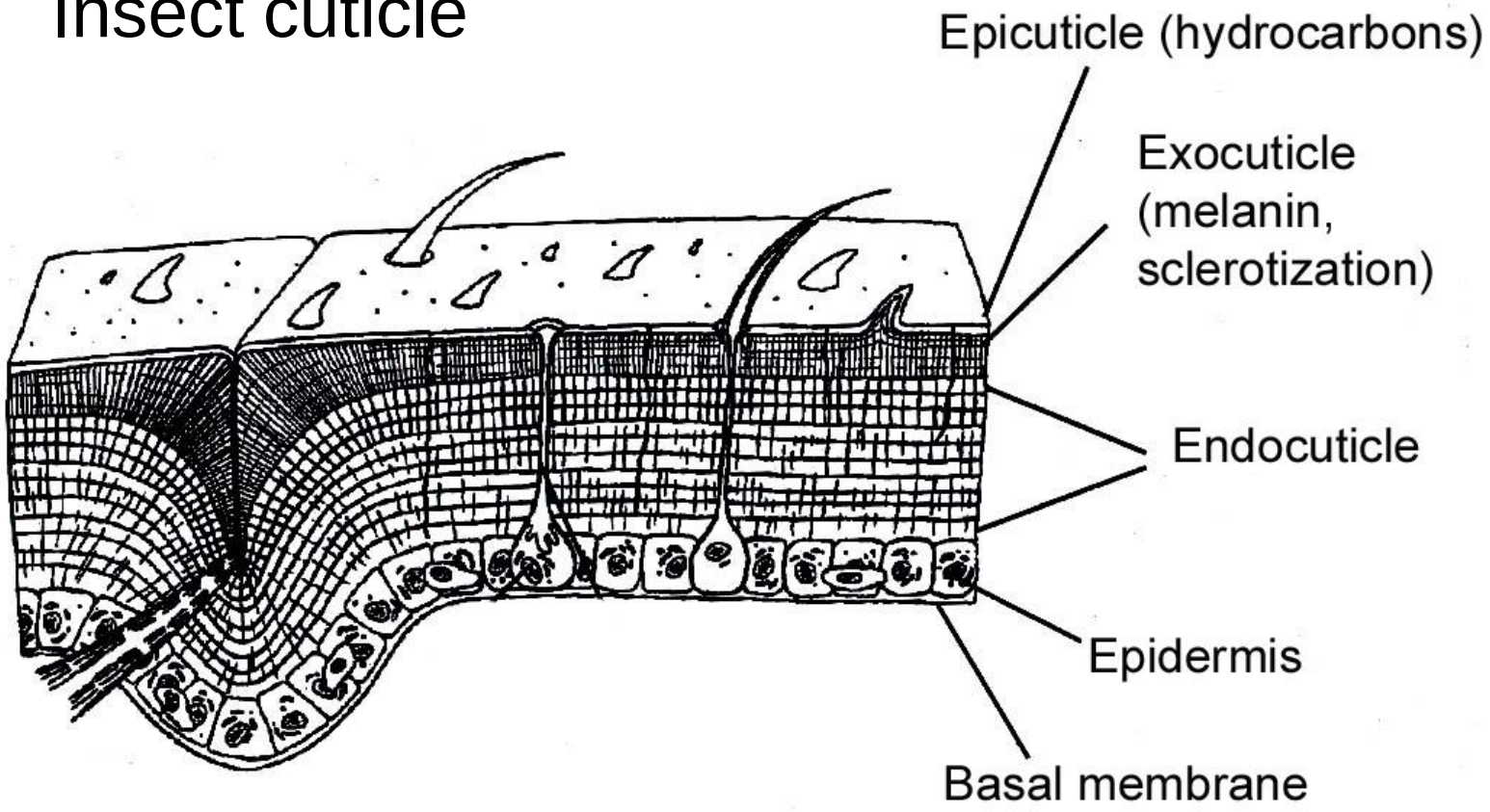
The roles of the ant's cuticle: protection against desiccation, nestmate and species recognition, trap for pollutants

Alain Lenoir

Emeritus Professor
IRBI, Institut de Recherche sur la Biologie de l'Insecte
UMR CNRS 7261
Université François Rabelais
Tours, France

Insect cuticle

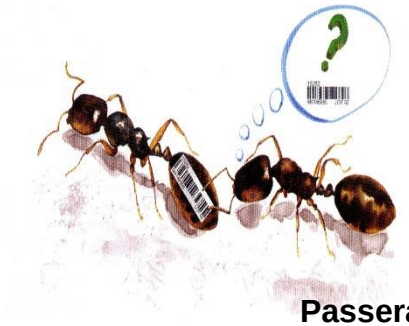


Insect cuticle is generally considered to be a barrier against the environment and protects against desiccation
In social insects, cuticular lipids also play a communication role

The roles of the ant's cuticle

1- Protection against desiccation and parasites

2- Species recognition



Passera Aron 2005

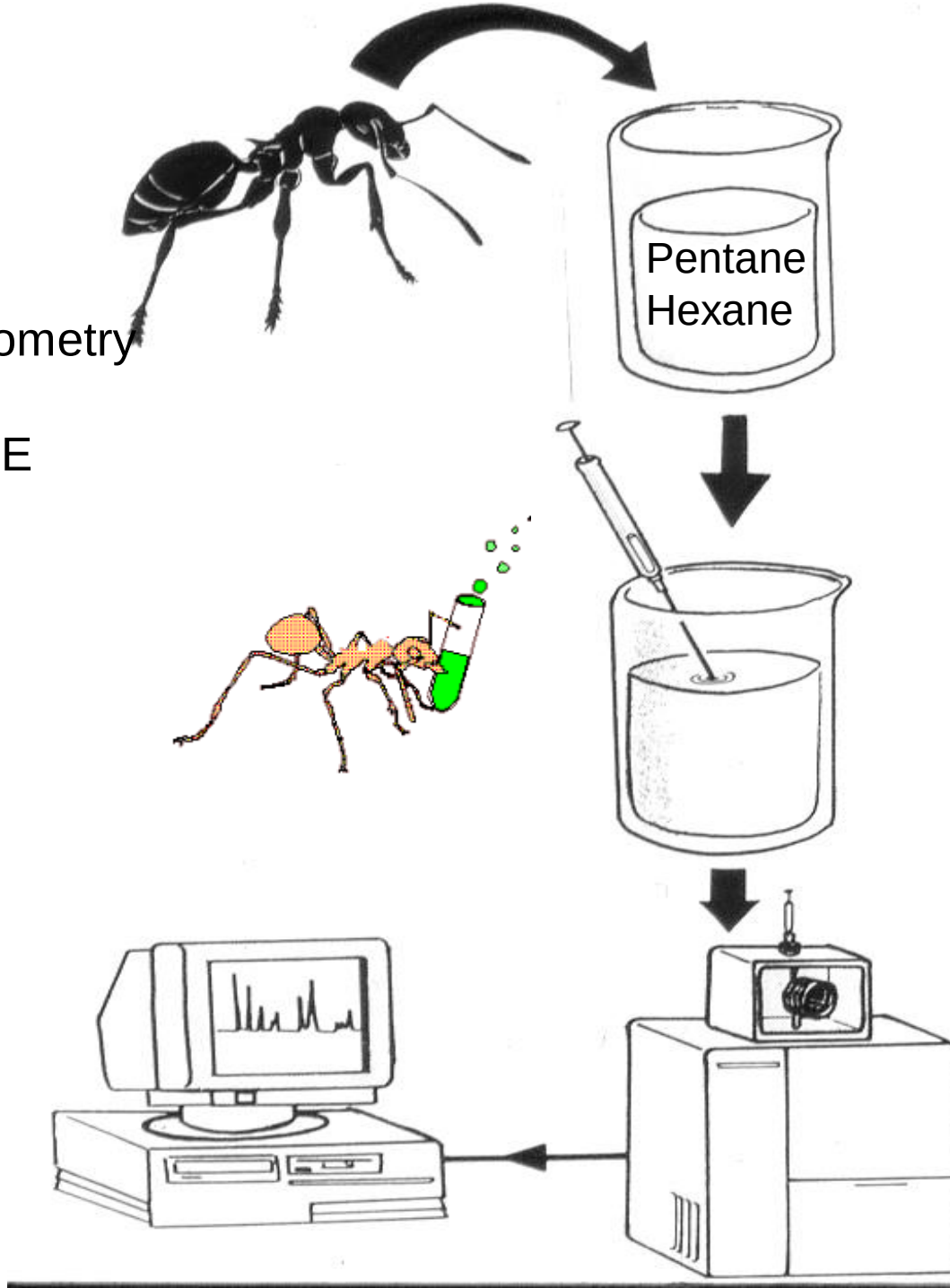
3- Nestmate recognition

3- Trap for pollutants

Tools

Gas chromatography Mass spectrometry

Solid Phase Micro Extraction SPME



Substances removed by the solvant (cuticle, glands)

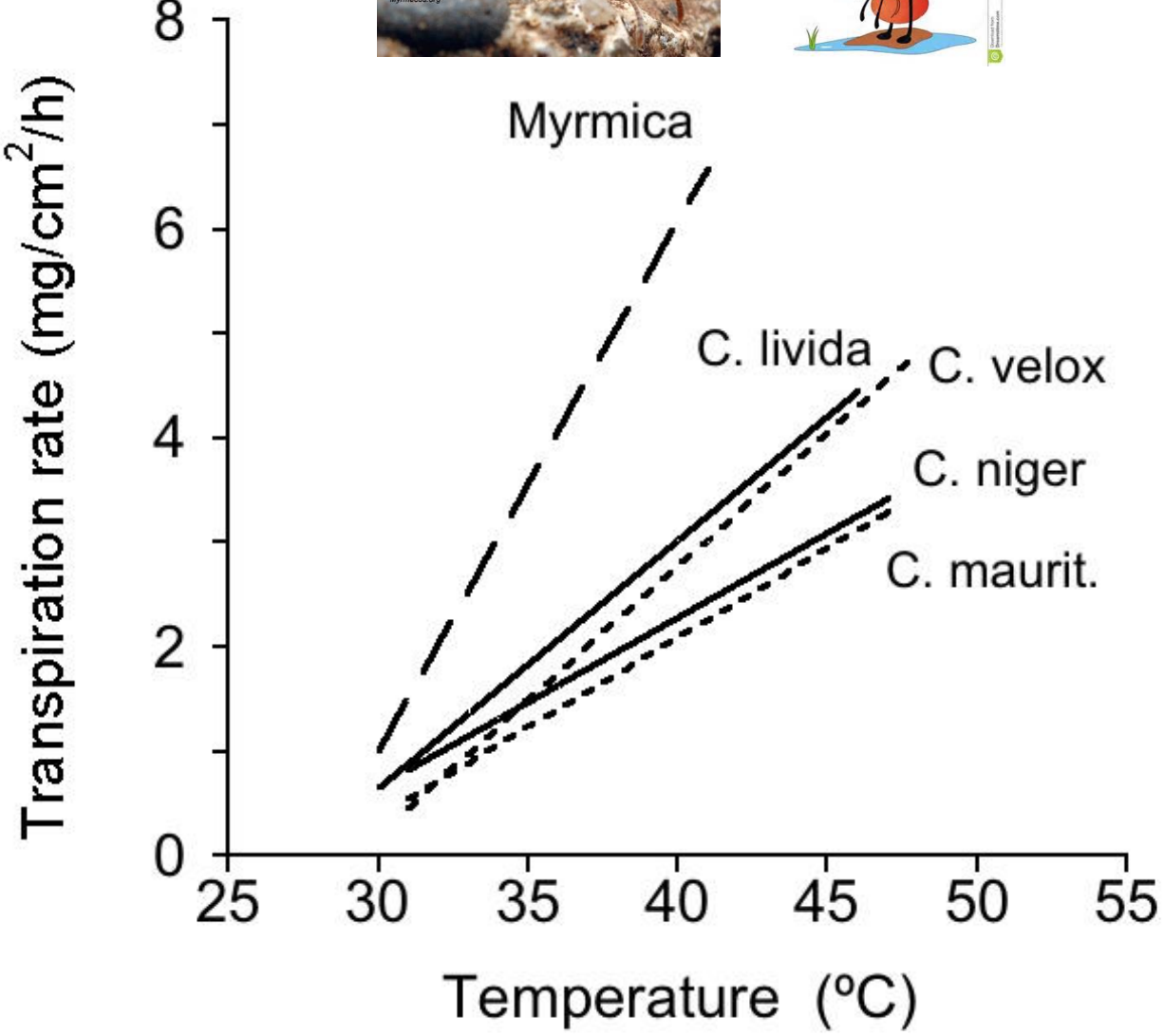
- Hydrocarbons
- Fatty acids
- Terpenes, sterols, alkaloids, acetates, alcohols…
- But not proteins

Protection against desiccation

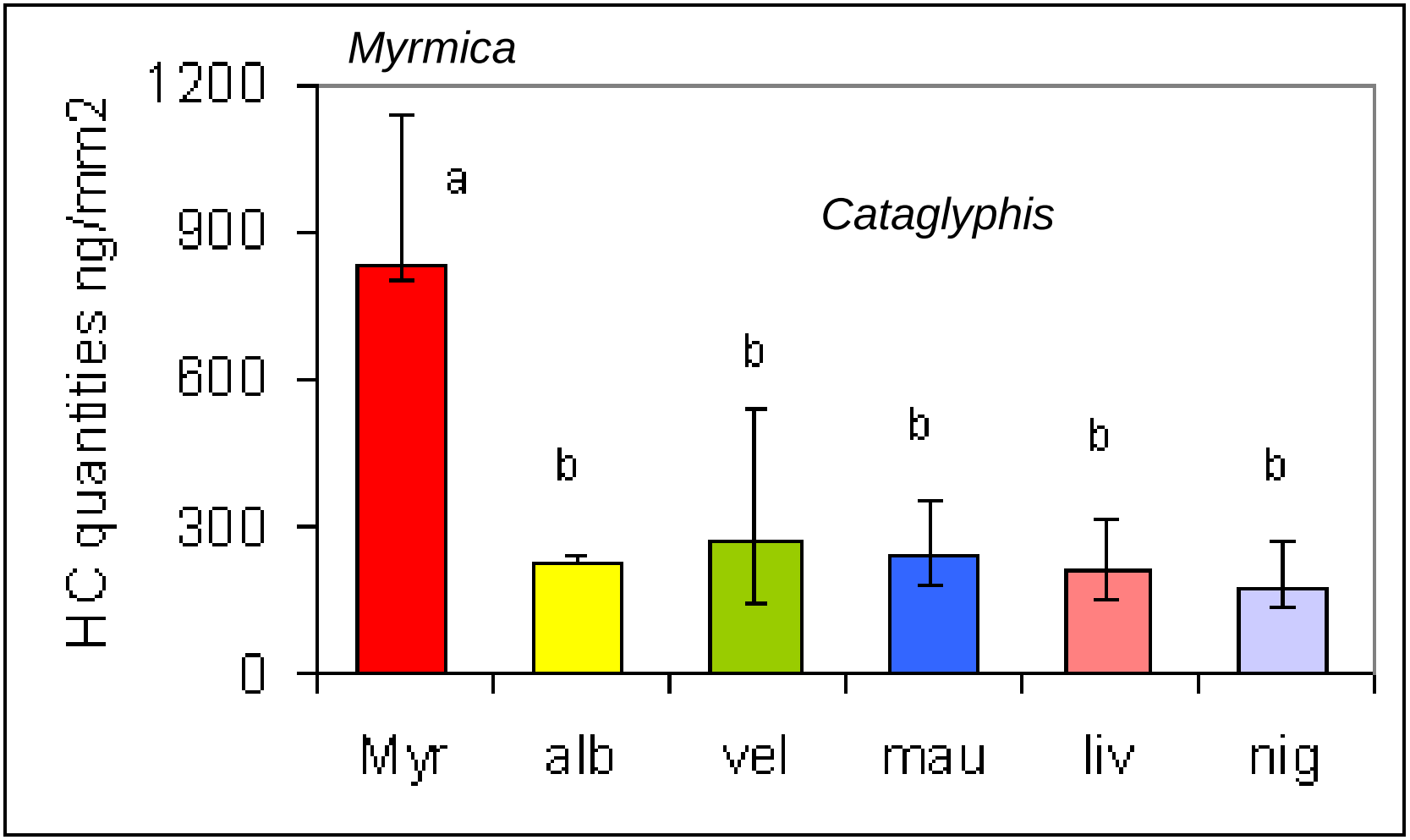
Composition and quantity of HCs appears to play a role in the desiccation resistance of insects with melanin

Foragers have more alkanes in ants

Desiccation stress -> increase in total amount of HCs with increase in chain length (scorpions)



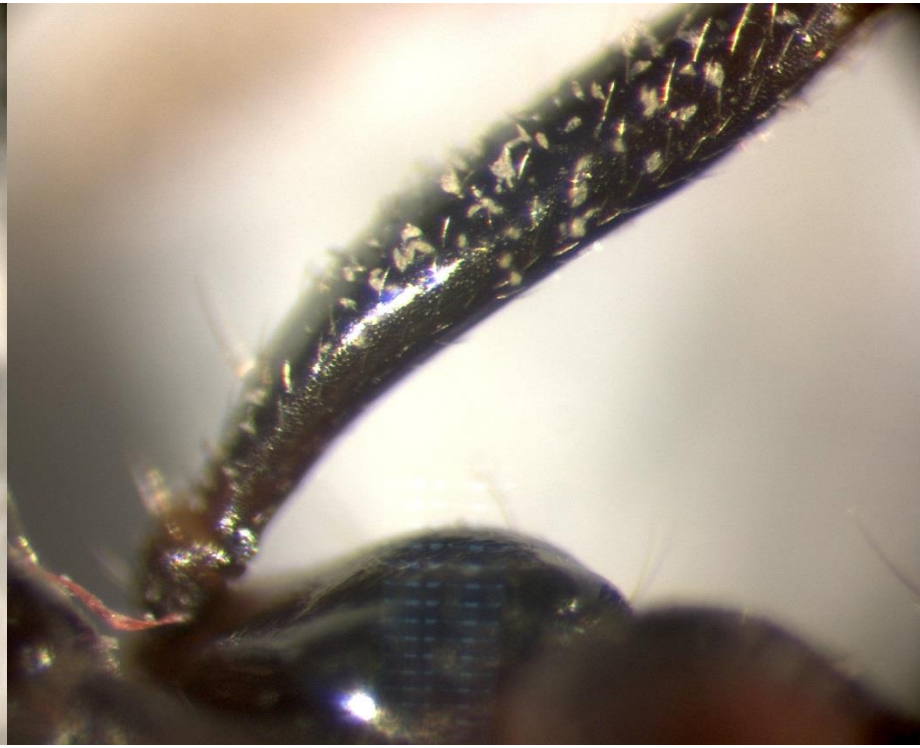
Lenoir et al 2009



Lenoir et al 2009

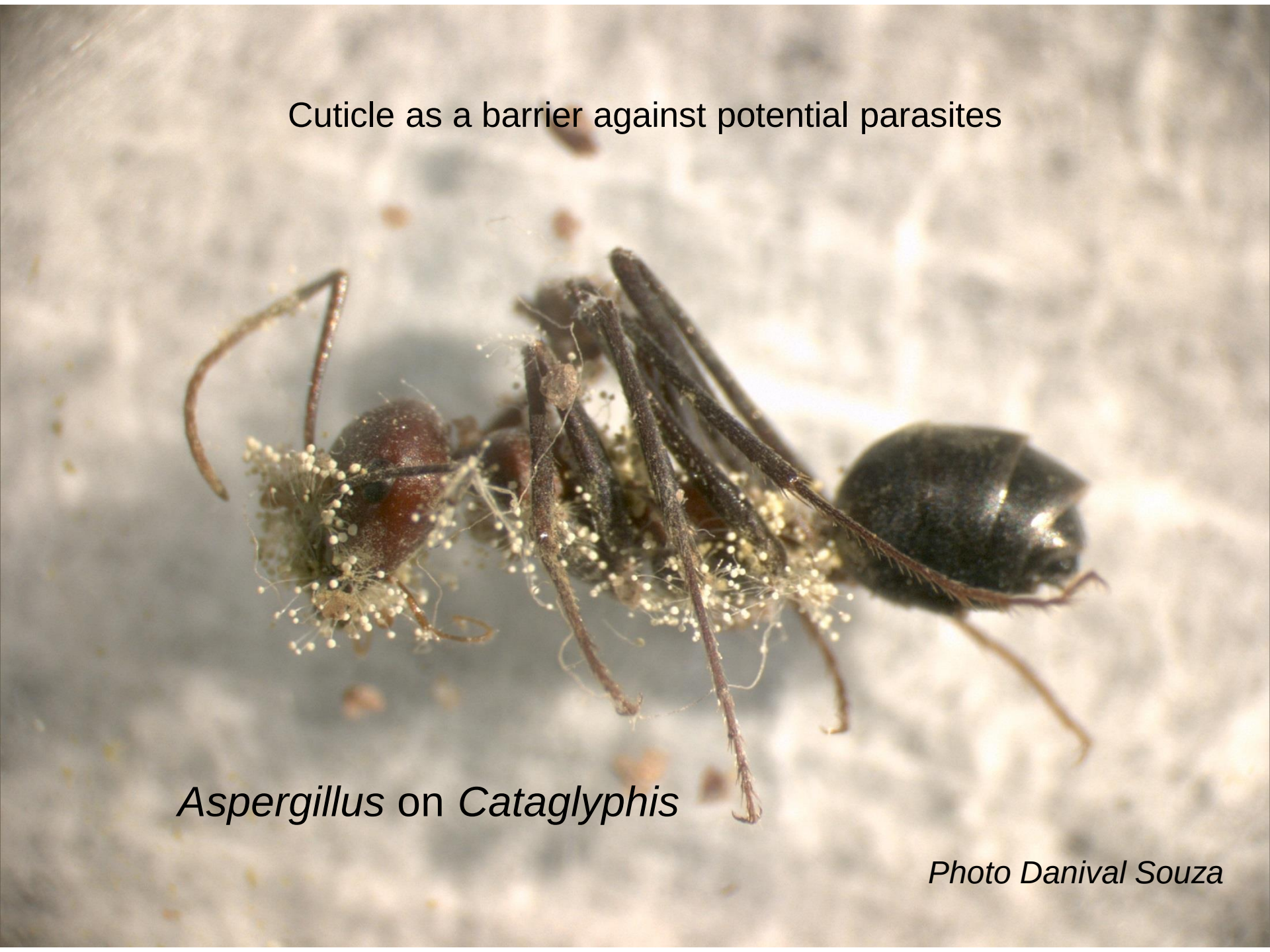
Adaptation to arid environments in ants is not related to hydrocarbon quantities on the cuticle but more on the relative composition: absence of alkenes, saturated hydrocarbons with longer chains.

Cuticle as a barrier against potential parasites



Rickia lenoirii (Laboulbeniales) ectoparasitic on *Messor*

Cuticle as a barrier against potential parasites



Aspergillus on *Cataglyphis*

Photo Danival Souza

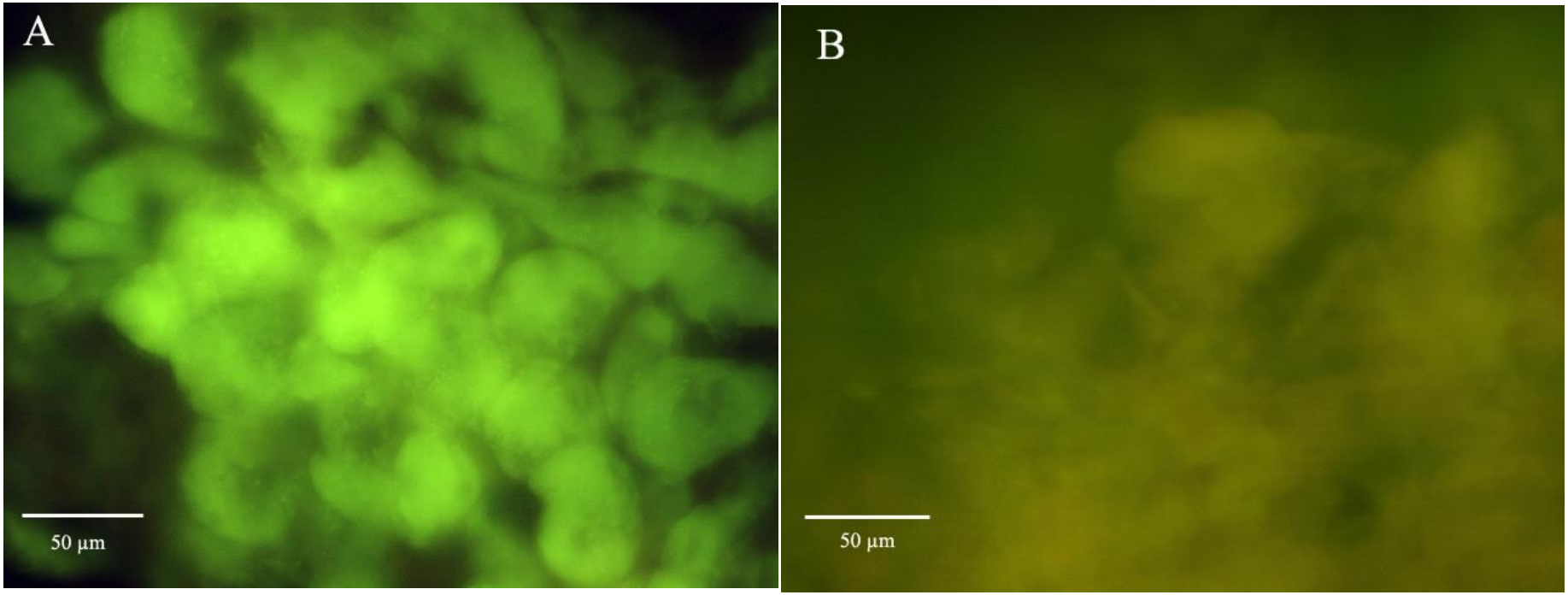
Cuticle as a barrier against potential parasites

Danival Souza Thesis 2008

Camponotus fellah

Bacteria discovered in *Camponotus ligniperdus* (Blochmann, 1892), live in specialised cells (bacteriocytes)

Antibiotic treatment (rifampicin)



FISH with a Blochmannia specific probe in the midgut of a *C. fellah* worker

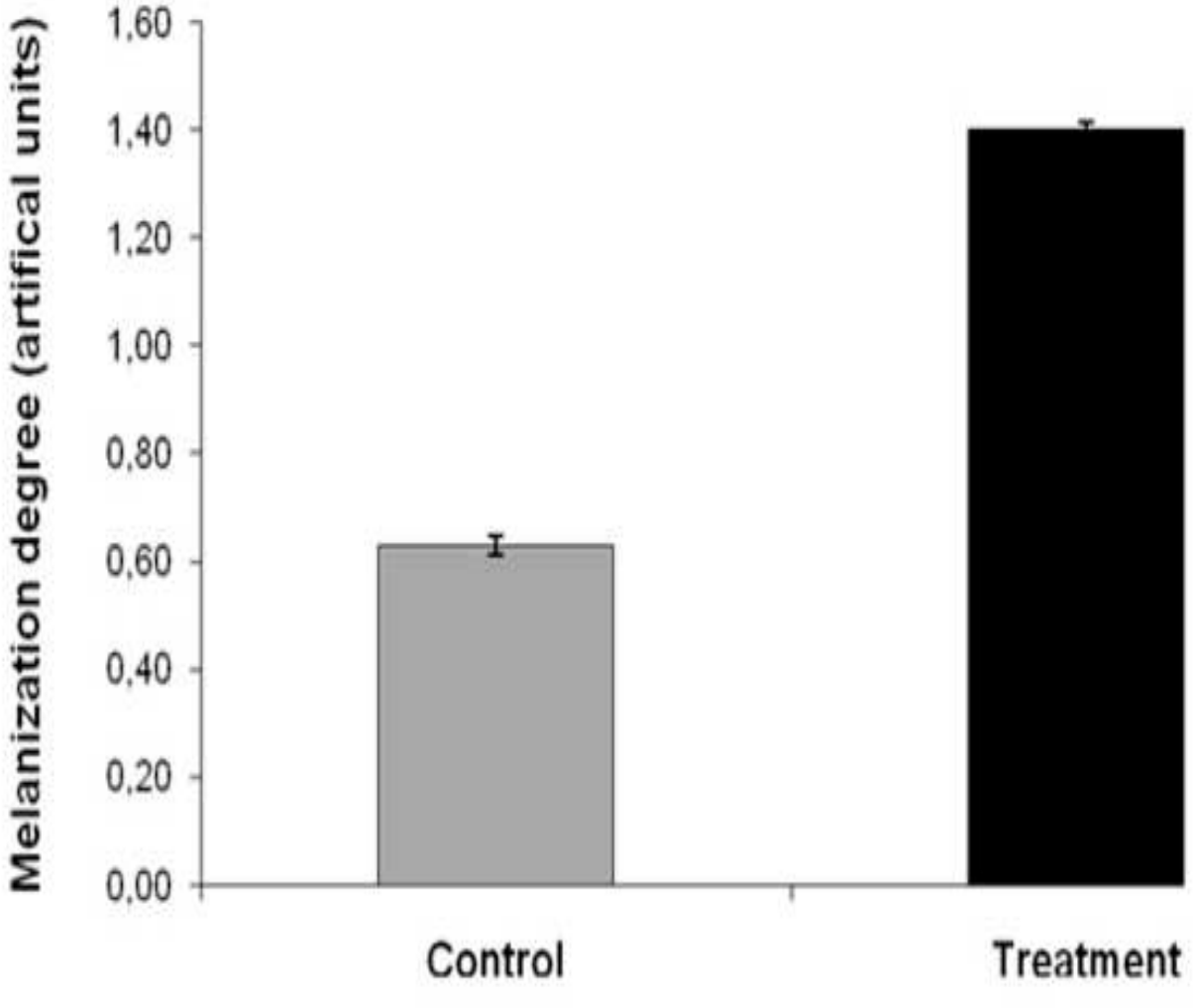
- left: the bacteria in green

- right: no bacteria after antibiotic treatment

Role of bacteria

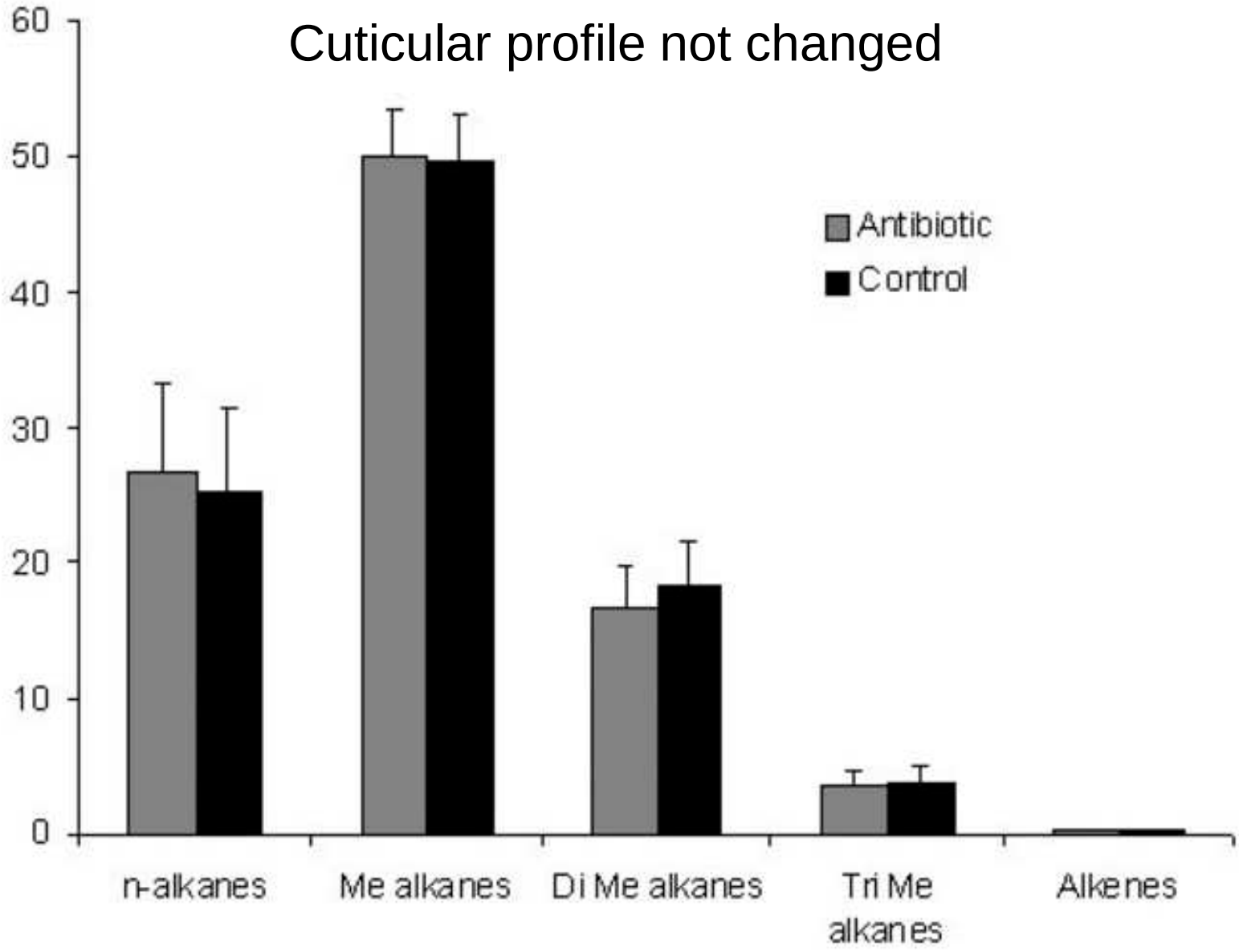
- Role in nutrition *(Feldhaar et al 2007)*
- Development of young colonies favored by the bacteria: advantage in the competition (Nutritional effect?) *(Souza et al. 2009)*
- The quantity of bacteria favors the encapsulation response: role in the immune system of the host Protection against parasitoids attacks? *(Souza et al. 2009)*
- **Other role?**

Melanization degree of the cuticule increase after antibiotic treatment



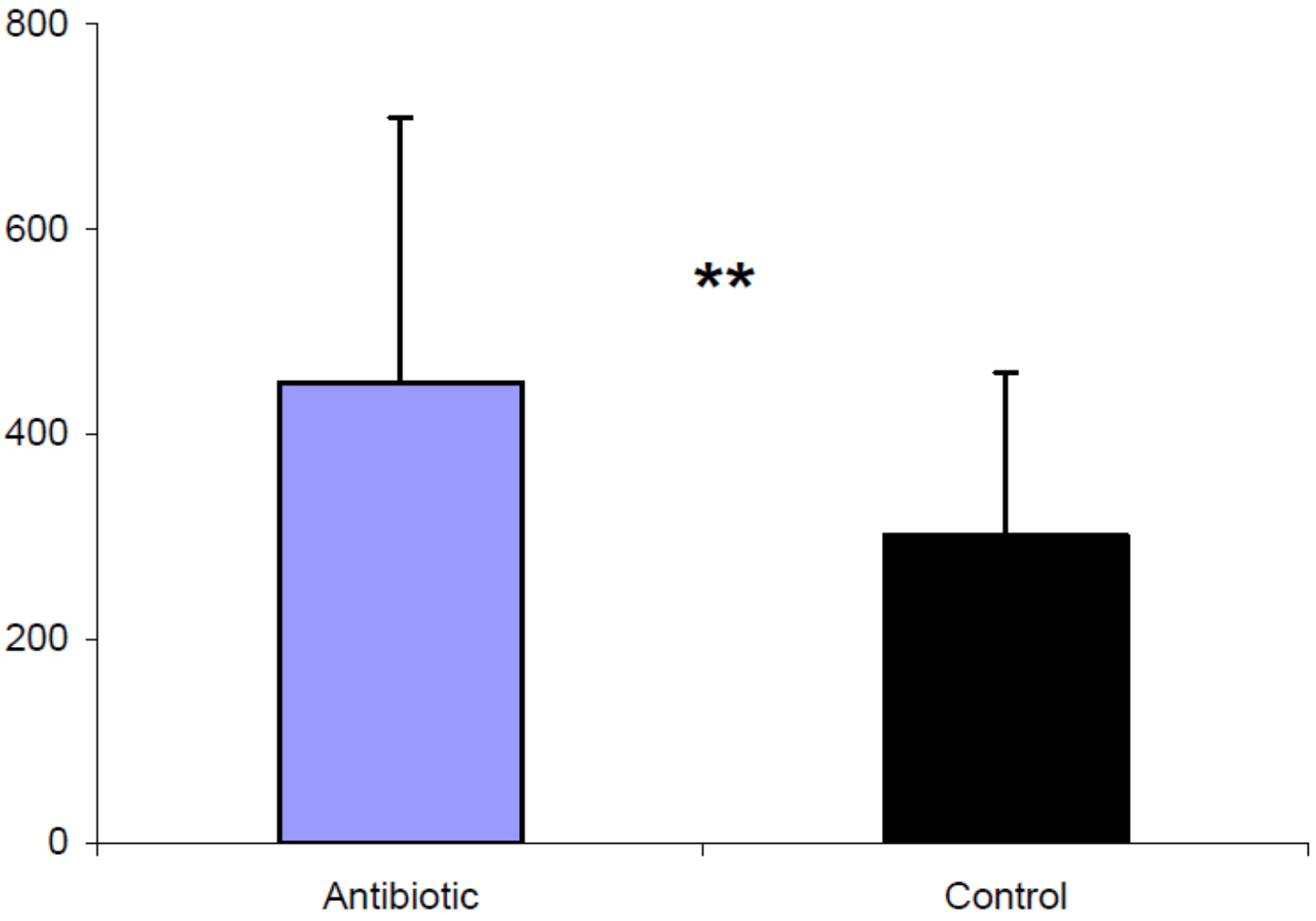
(De Souza, ,Lenoir 2011)

Cuticular profile not changed



(De Souza, ,Lenoir 2011)

Hydrocarbon quantities (ng)

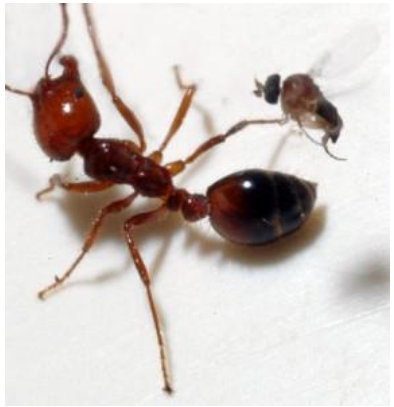


(De Souza, ,Lenoir 2011)

New role of endosymbiont bacteria

Increase of cuticular hydrocarbons quantity and melanization in the absence of bacteria

→ Absence of bacteria = stress due to immunity deficit? → protection enhanced against pathogens (Phoridae for ex.)?



(De Souza, ,Lenoir 2011)

The roles of the ant's cuticle:

1- Protection against desiccation and parasites

2- Species recognition

3- Nestmate recognition

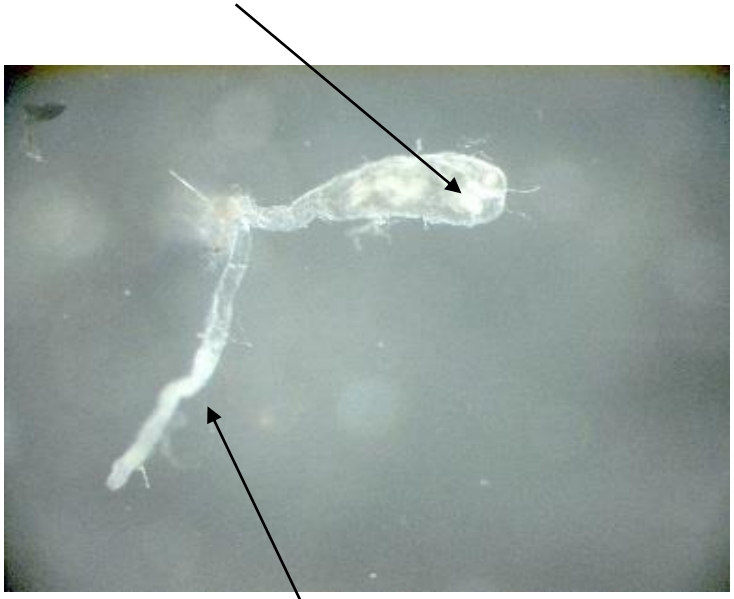
4- Trap for pollutants

Glandular composition in *Aphaenogaster*

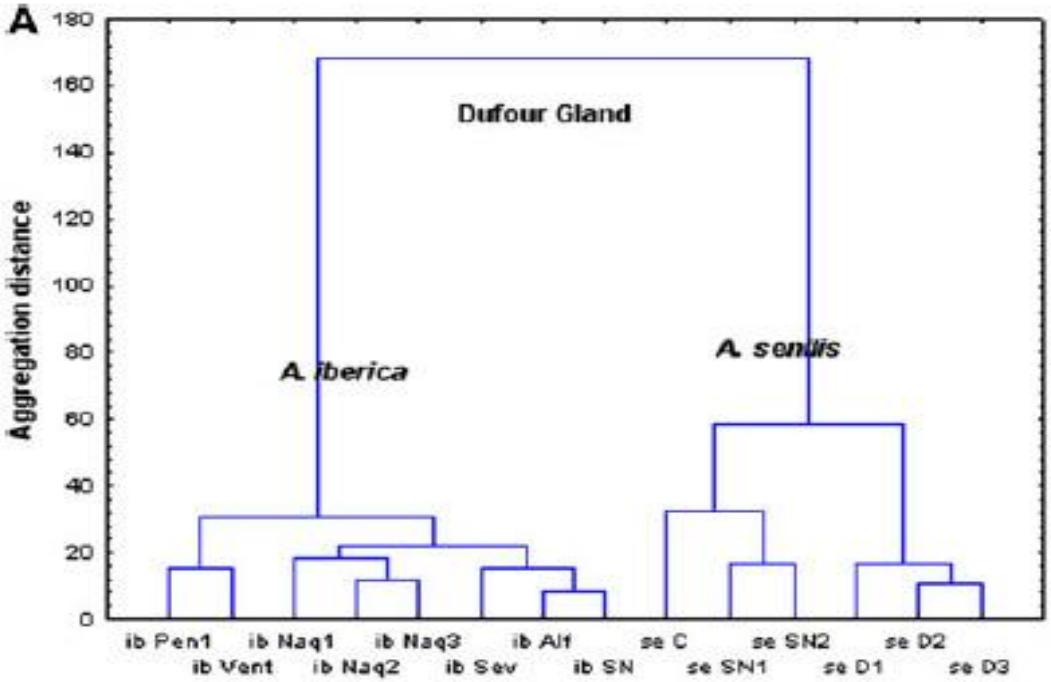
A. senilis

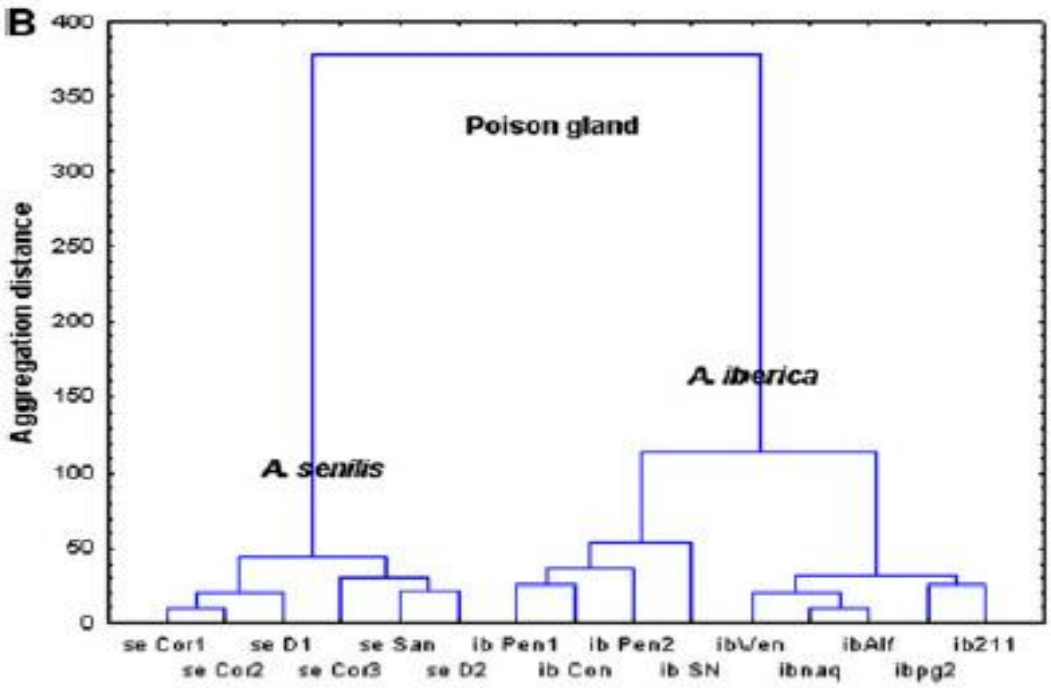
A. iberica

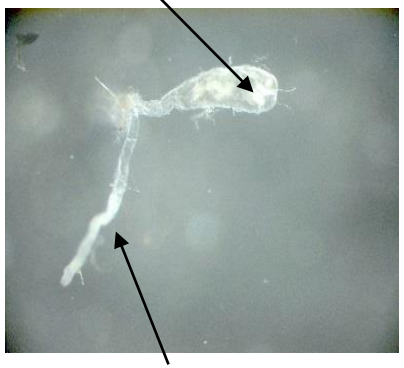
Poison gland



Dufour gland

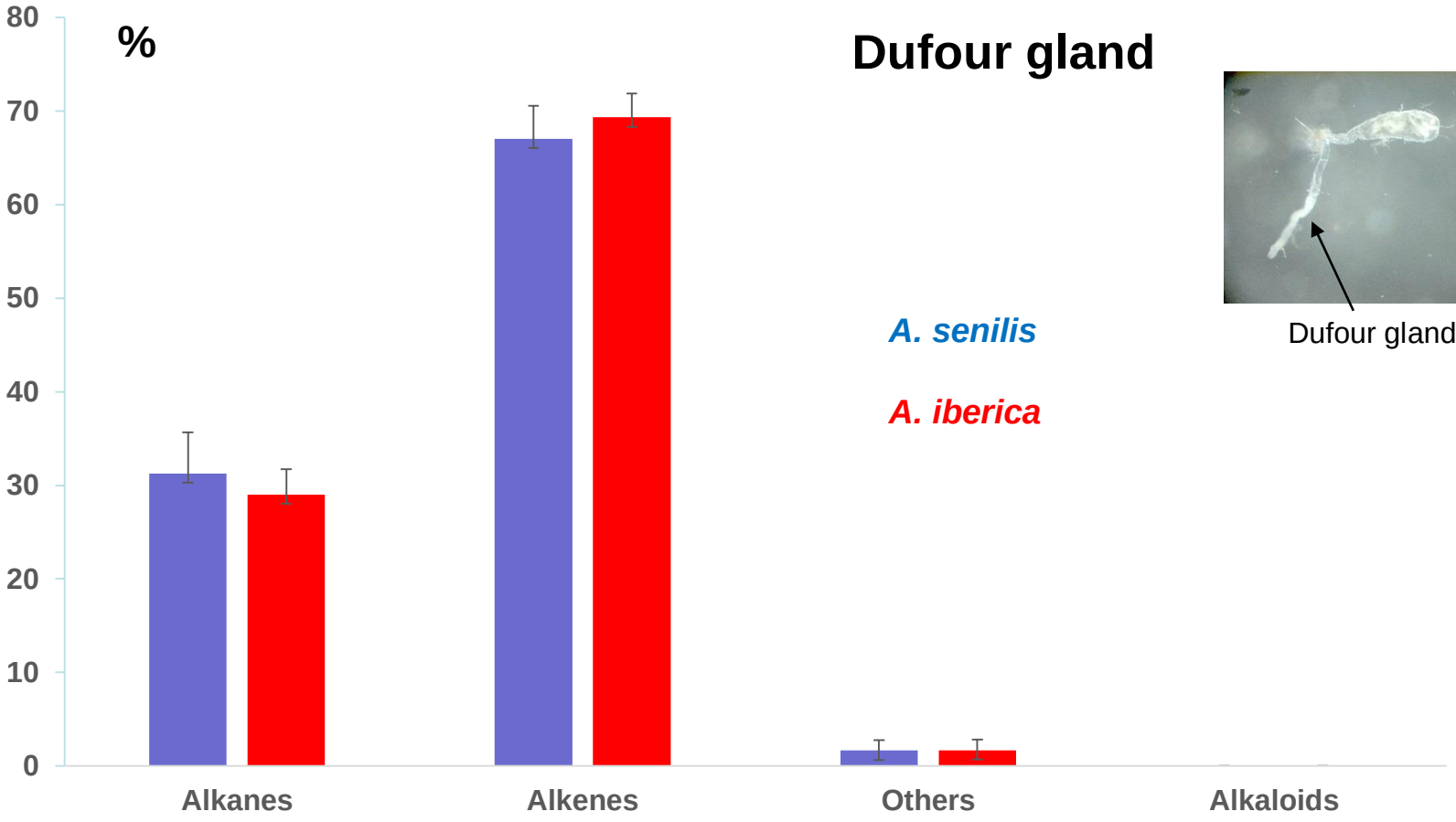






Lenoir,…, Hefetz 2011

Glandular composition in *Aphaenogaster*



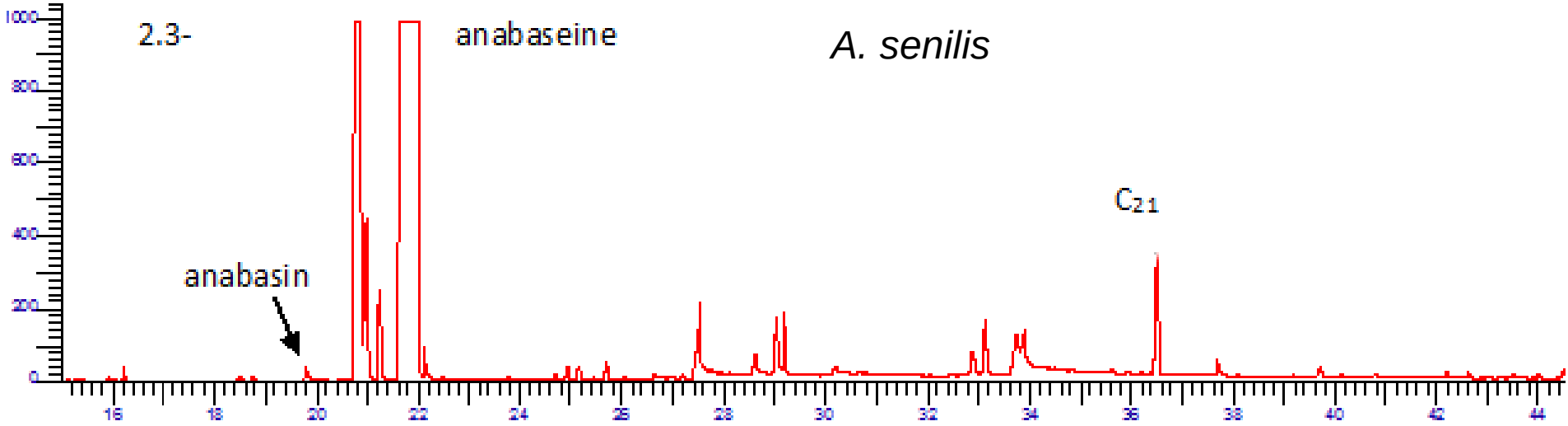
Dufour gland

Lenoir,…, Hefetz 2011

Poison gland of *Aphaenogaster*

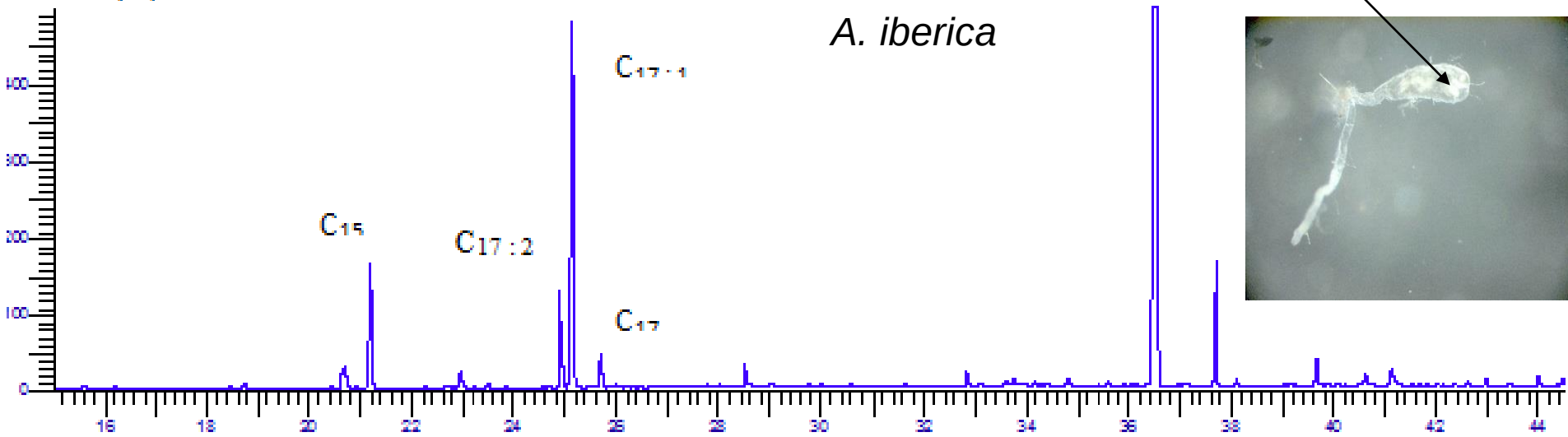
(C)

A. senilis



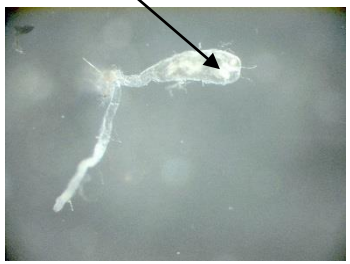
(D)

A. iberica



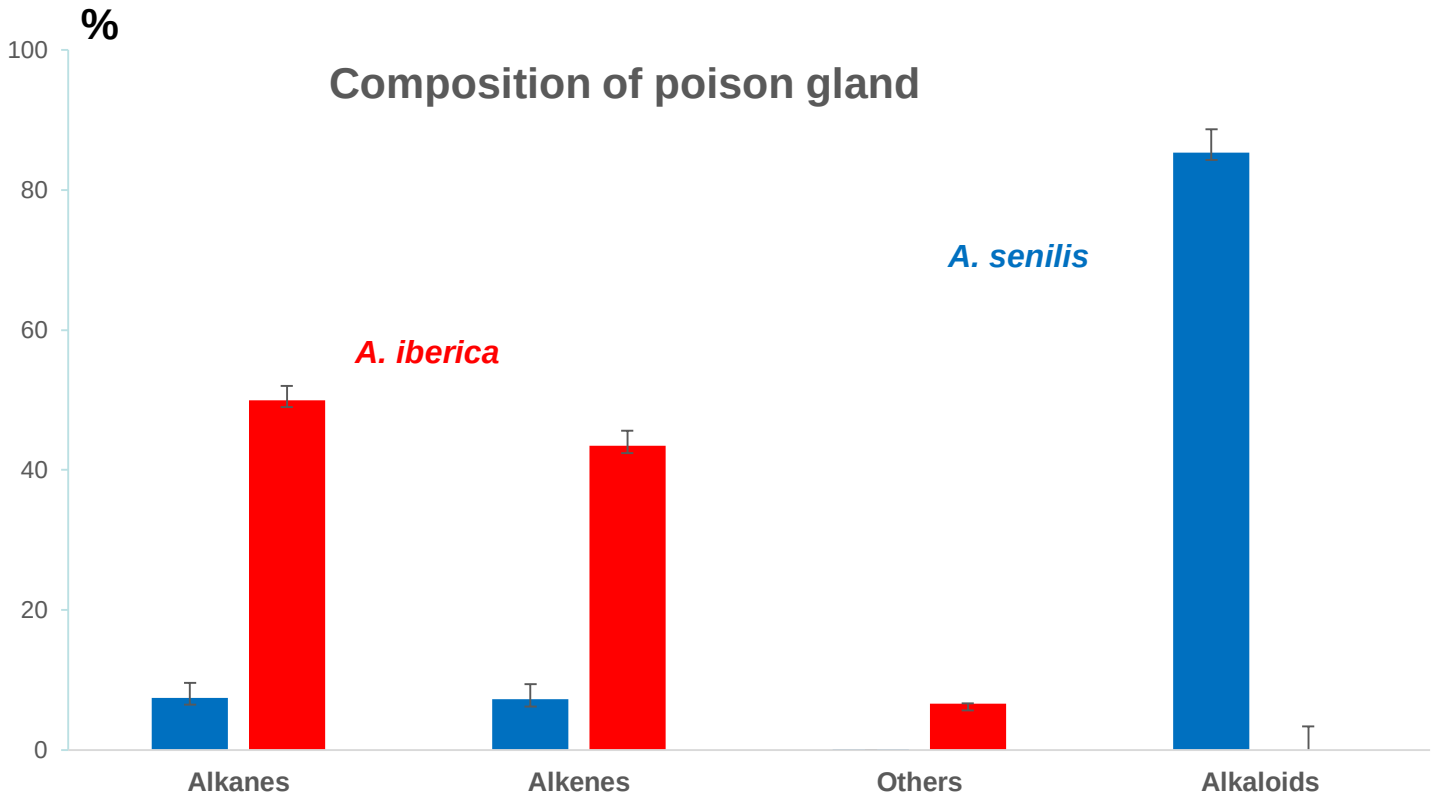
Poison gland

Alkaloids in the poison gland



Aphaenogaster senilis: alkaloids mainly anabaseine

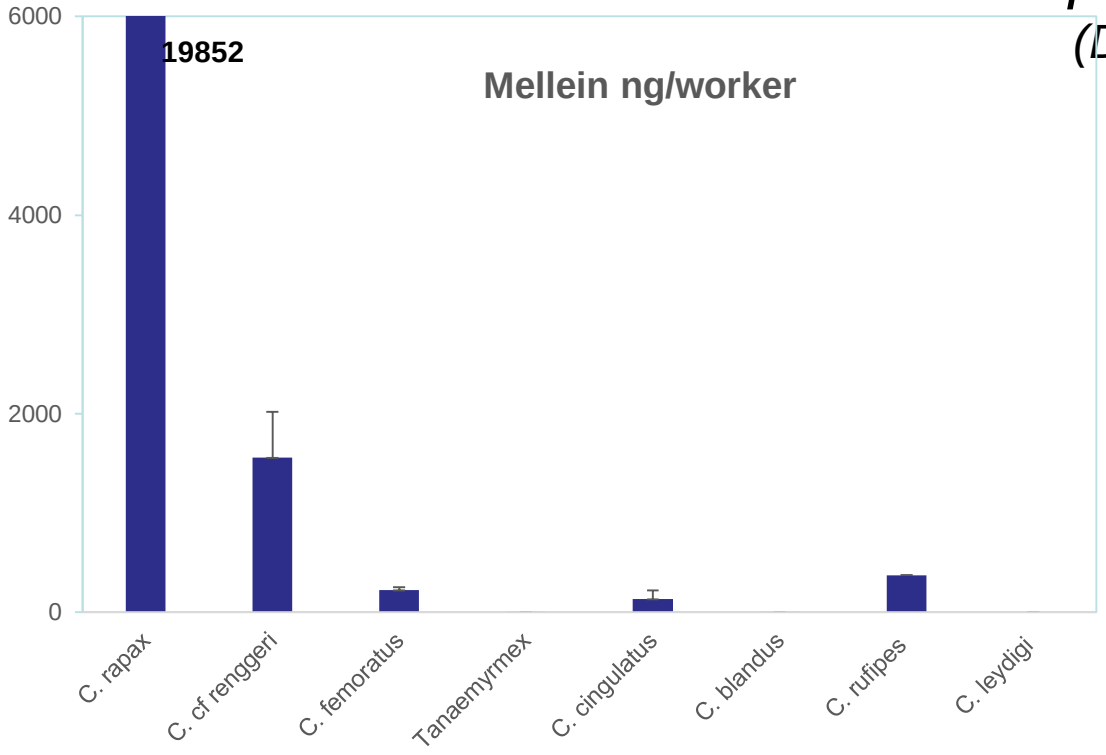
Aphaenogaster iberica: no alkaloids



Lenoir,…, Hefetz 2011

Camponotus from French Guyana

(Determination Jacques Delabie)



Camponotus rapax
Until 100µg/ant
Predator

Mellein: dihydroisocoumarin produced by *Aspergillus* and some plants
Biological activities including antibacterial, antimalarial, antifungal, and anticancer effects

In *Camponotus* from mandibular gland or rectum?

Camponotus from French Guyana

, 14-Nov-2013 + 09:37:02

Campo45CR-14nov — Scan EI+

C. cingulatus

3% alkenes C31:1 C36 DiMeC35 DiMeC37

Campo25-20nov — Scan EI+ TIC 6.61e8

C. femoratus

33% alkenes X DiMeC37 Cxx:1

Campo23a-14nov — Scan EI+ TIC 2.54e9

C. cf renggeri

C27:1 C29:1 55% alkenes

Campo21a-15nov — Scan EI+ TIC 6.84e8

C. rapax

58% alkenes C31:1 C33:1

Campo46A-18nov — Scan EI+ TIC 1.98e9

C. blandus

7% alkenes C31:1 DiMeC35 DiMeC37

100 / % / 0

19.81 20.31 20.81 21.31 21.81 22.31 22.81 23.31 23.81 24.31 24.81 25.31 25.81 26.31 26.81 27.31 27.81 28.31 28.81 29.31 29.81 30.31 30.81 Time

The roles of the ant's cuticle:

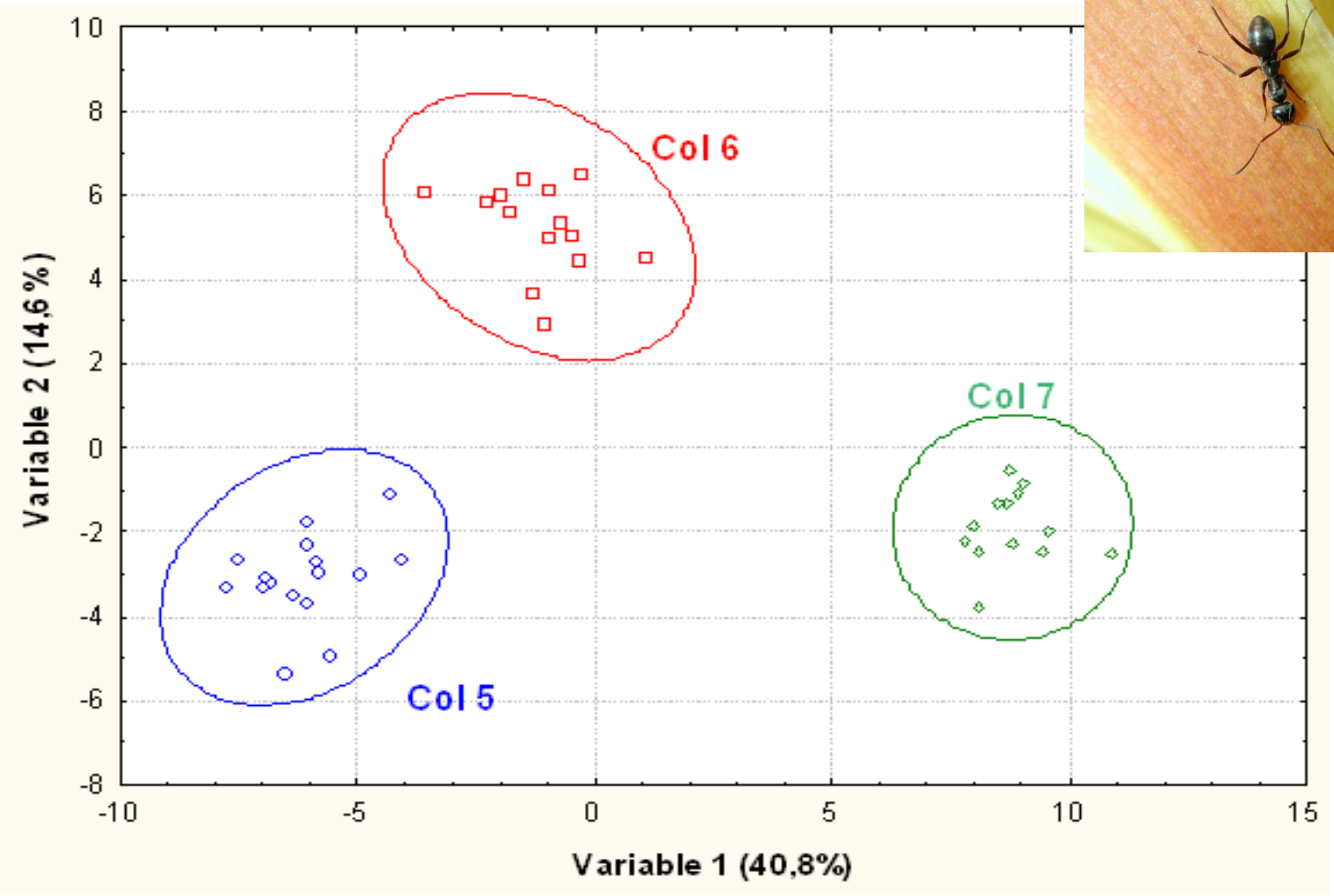
3- Nestmate recognition

Many correlative and experimental studies suggest the hydrocarbon fraction of the **cuticular lipids**

Hefetz and Donacimento talks

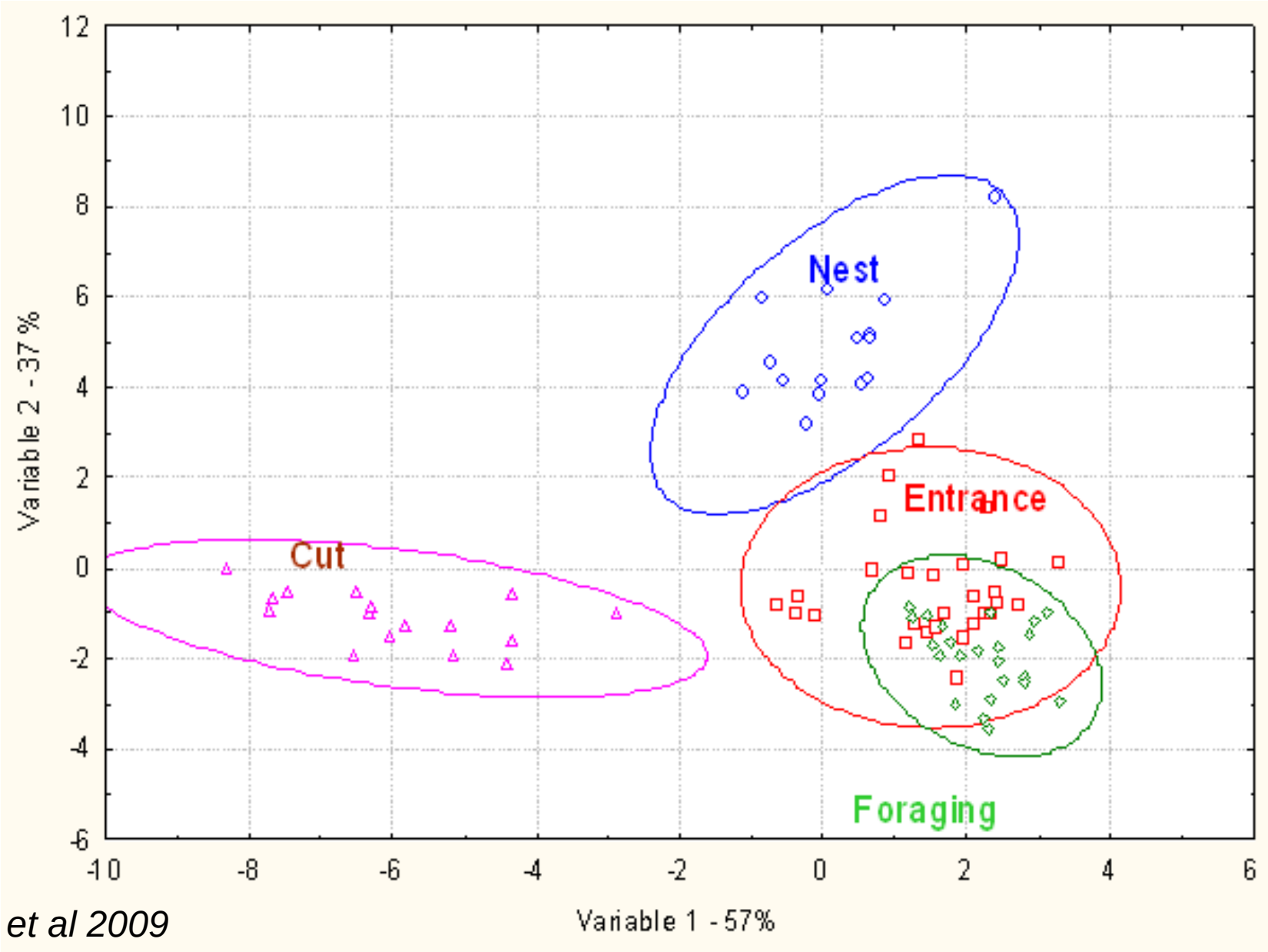
Passera Aron 2005

Discriminant analysis of *Lasius niger* colonies: Nestmate recognition



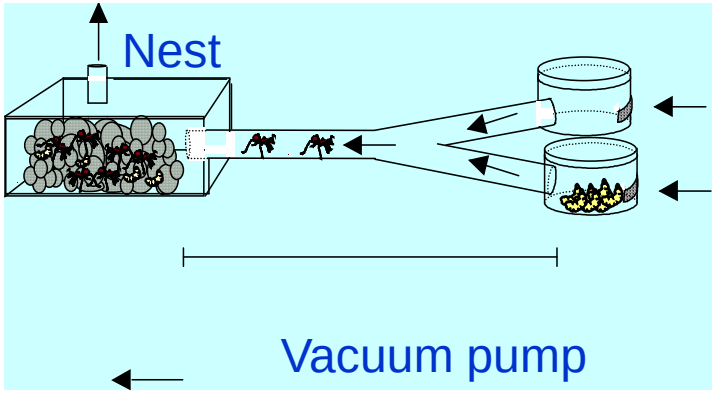
Lenoir et al 2009

HCs in *L. niger* (SPME)



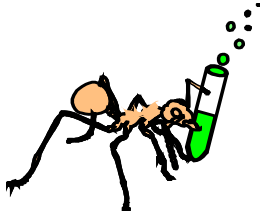
Lenoir et al 2009

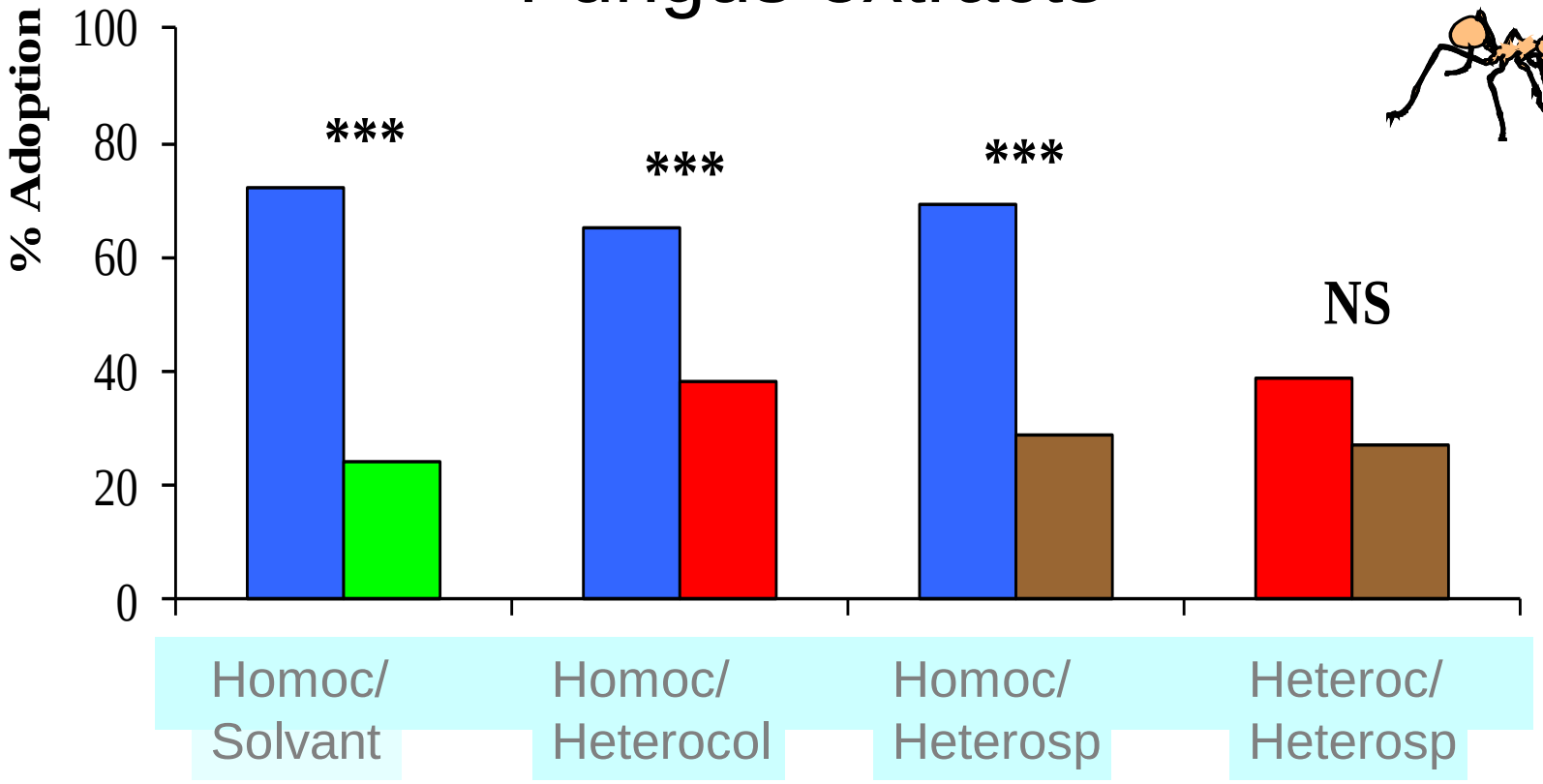
Colonial odor of fungus



Ana Maria Matoso Viana

Fungus extracts

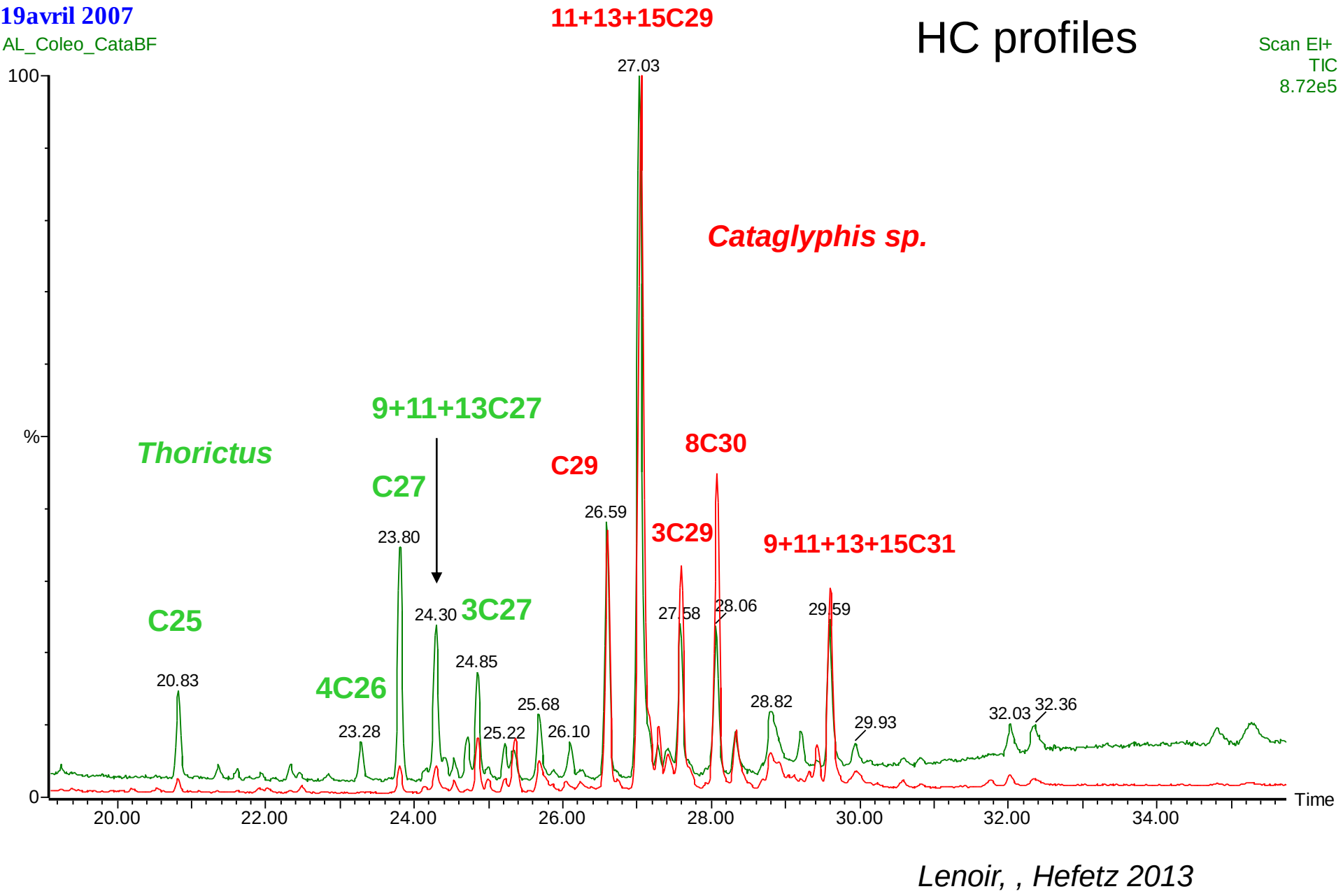




Homocol > Heterocol = Heterosp= Solvant

Viana & Lenoir, 2001


Colonial odor of guests and parasites
Thorictus
Cataglyphis viatica (Morocco)
Photo Fernando Amor (Sevilla)



Lenoir, , Hefetz 2013

Conclusions

Myrmecophiles are tolerated into the host colony due to chemical mimicry

They share the host colony odor but can be adopted in other colonies of the same species, but never in allospecific species

Sternocoelis beetles may synthesize the same hydrocarbons than their host
→coevolution?

Subcaste discrimination

Callows: very small quantity of HCs
= Chemical insignificance

Lenoir, , Hefetz 2001

The roles of the ant's cuticle:

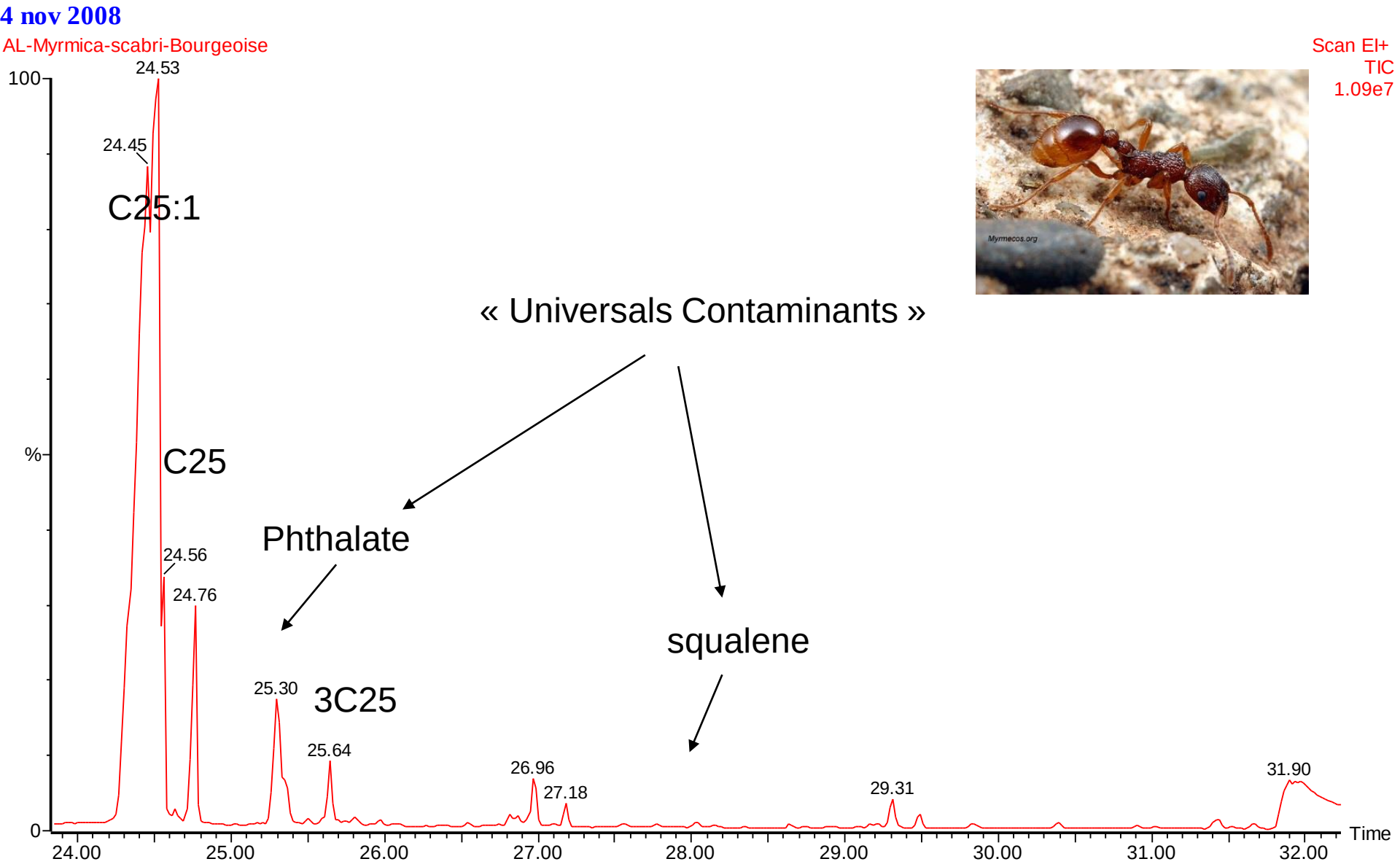
1- Protection against desiccation and parasites

2- Species recognition

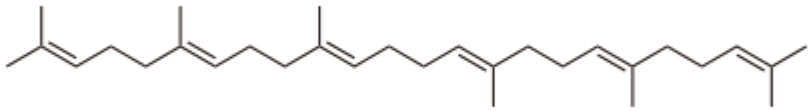
3- Nestmate recognition

4- Trap for pollutants

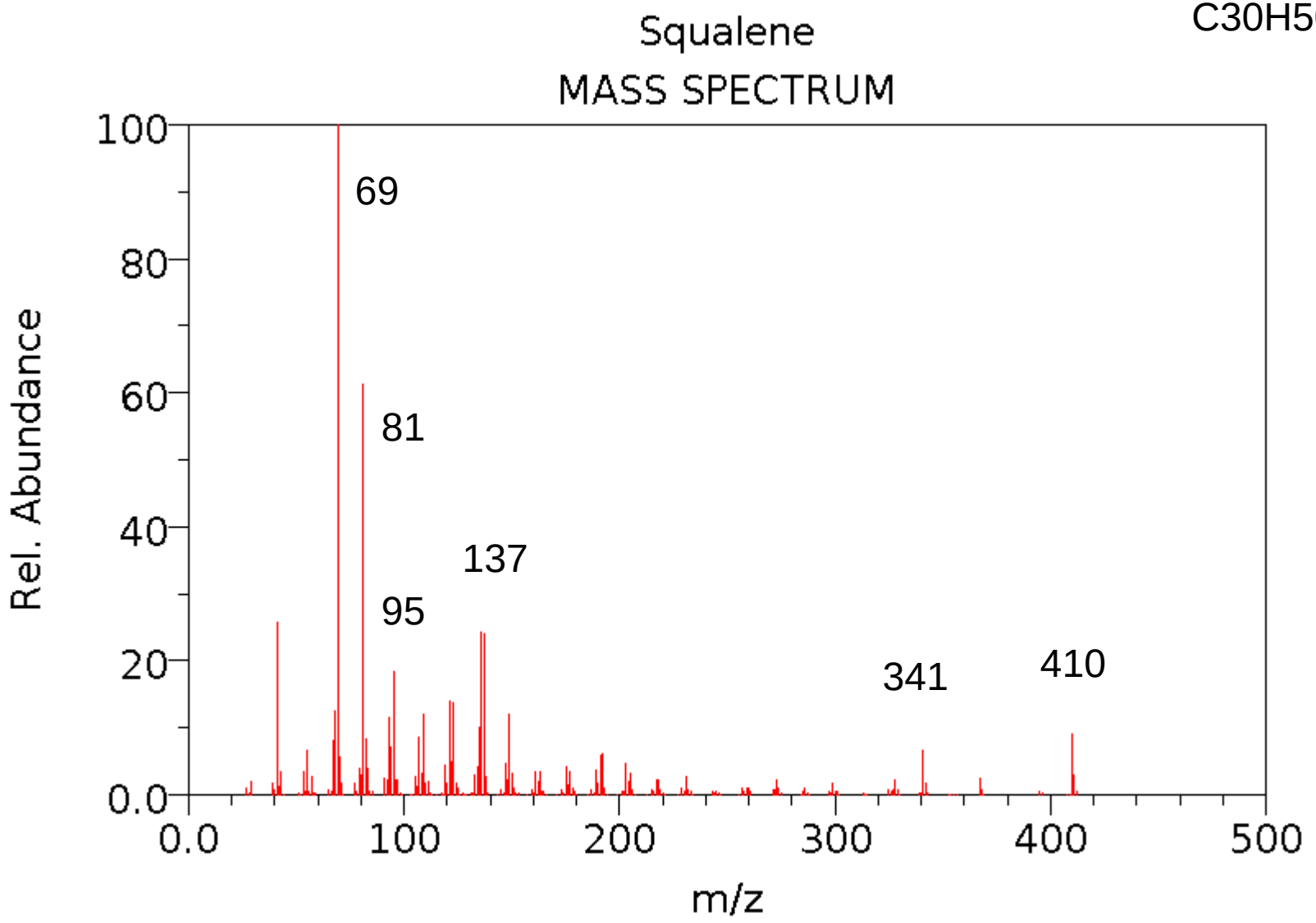
Pollutions in chromatograms



Squalene (C30 alkene)



C30H50, mw 410



NIST Chemistry WebBook (http://webbook.nist.gov/chemistry)

Squalene

Oils (sharks, olive)
Precursor of cholesterol in the liver
High quantities on human skin = attractant for ticks which concentrate it and use as defensive secretion

In vegetals

Not in invertebrates (insects without cholesterol)

Surfactant in cosmetics

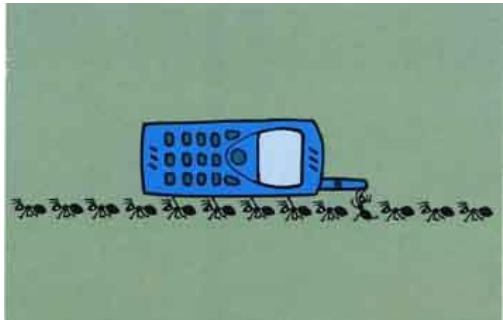
0.5 to 3% on ants: contamination by humans

Phthalates

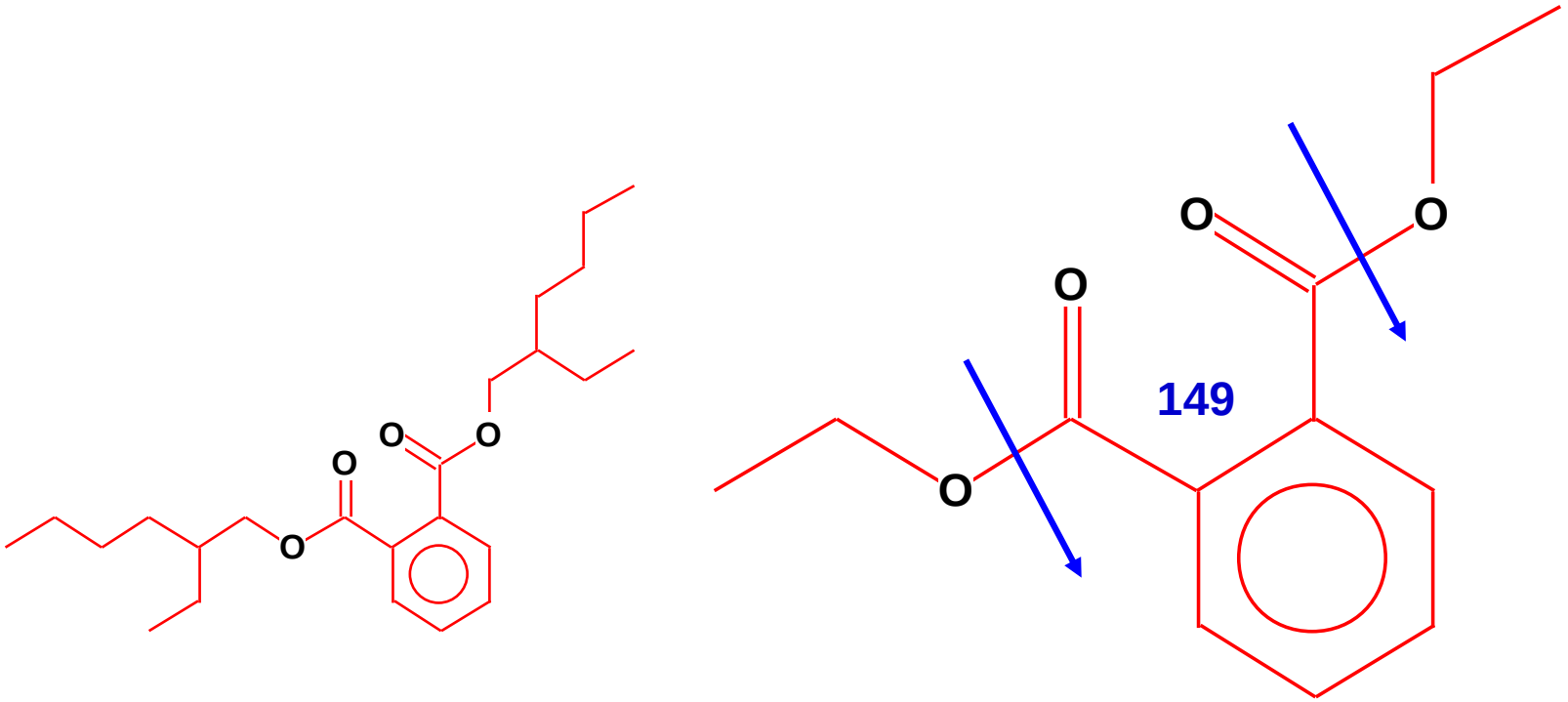
- used in great quantities to make PVC plastics more souple, less crushing

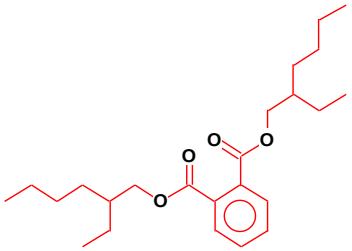
- small phthlates molecules as solvent

- but soft link with plastic → released in the environment, more when plastic is old

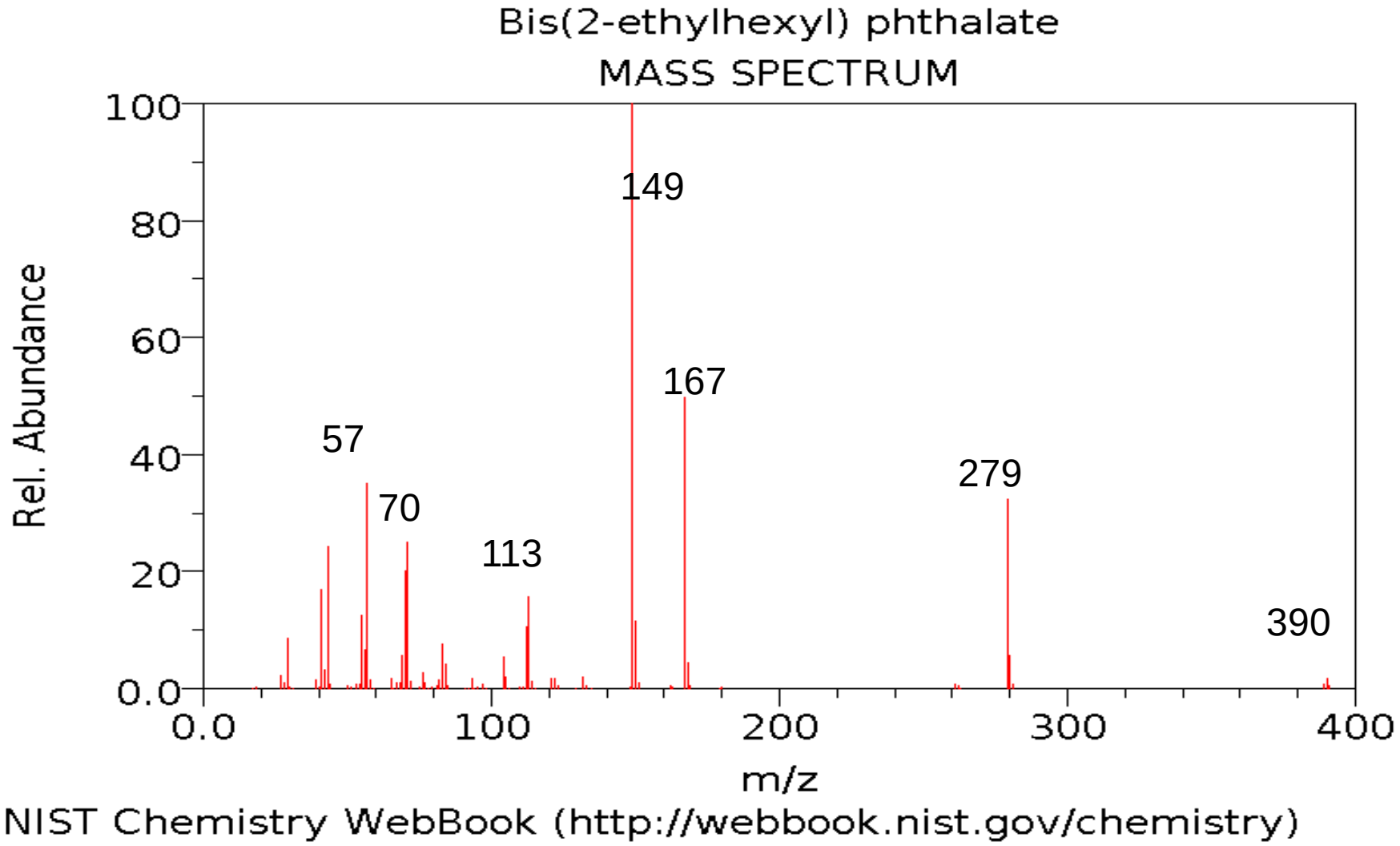


Phthalates in chromatograms

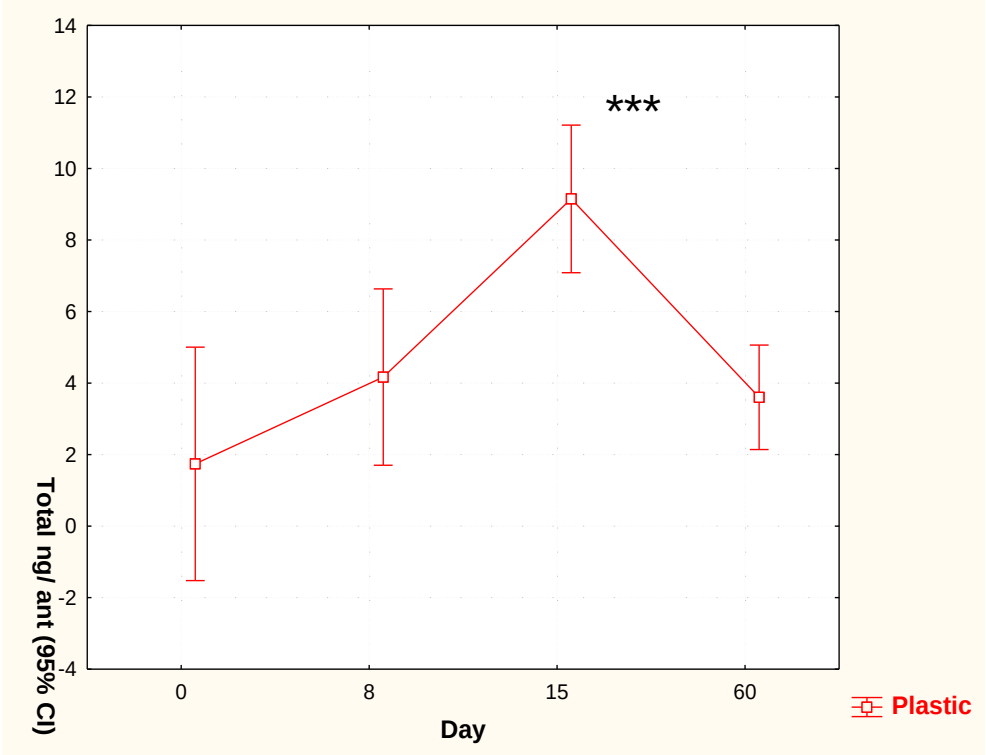




DEHP = Di Ethyl Hexyl Phtalate)



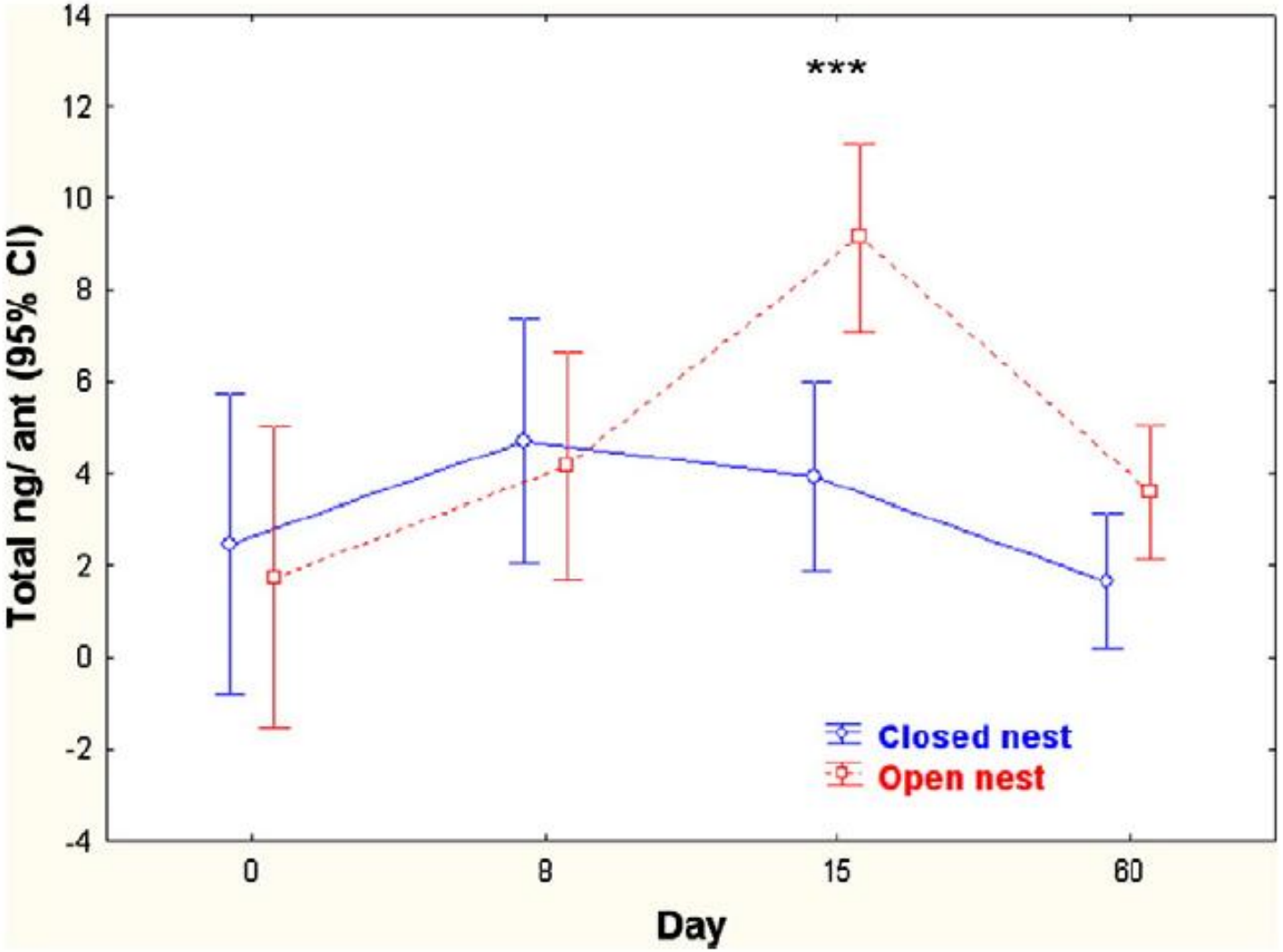
Origine of the phtalates: plastic boxes?



But no phtalates in this plastic!

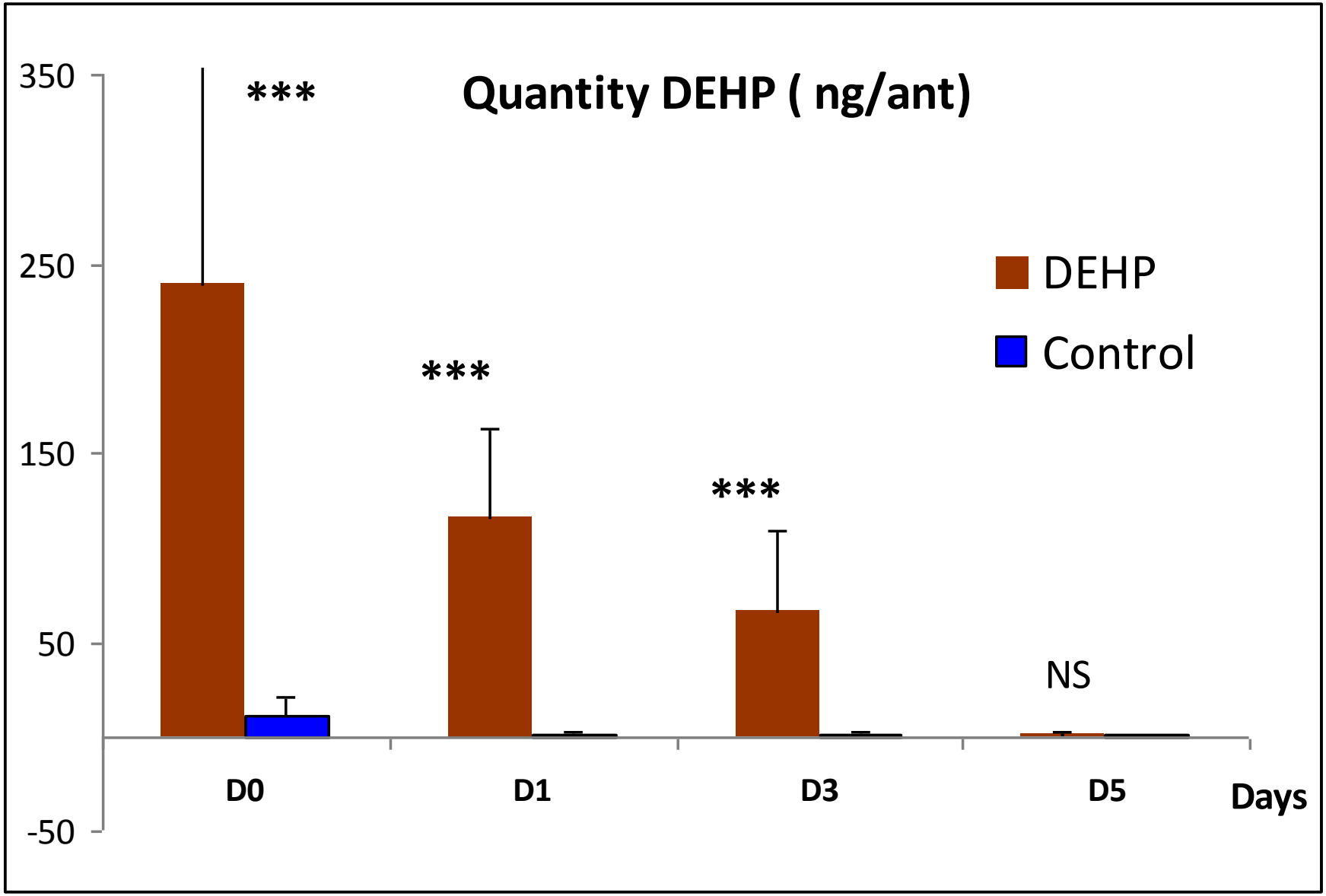
Lenoir et al 2012

Accumulation of phthalates on ants reared in open nests



Lenoir et al 2012

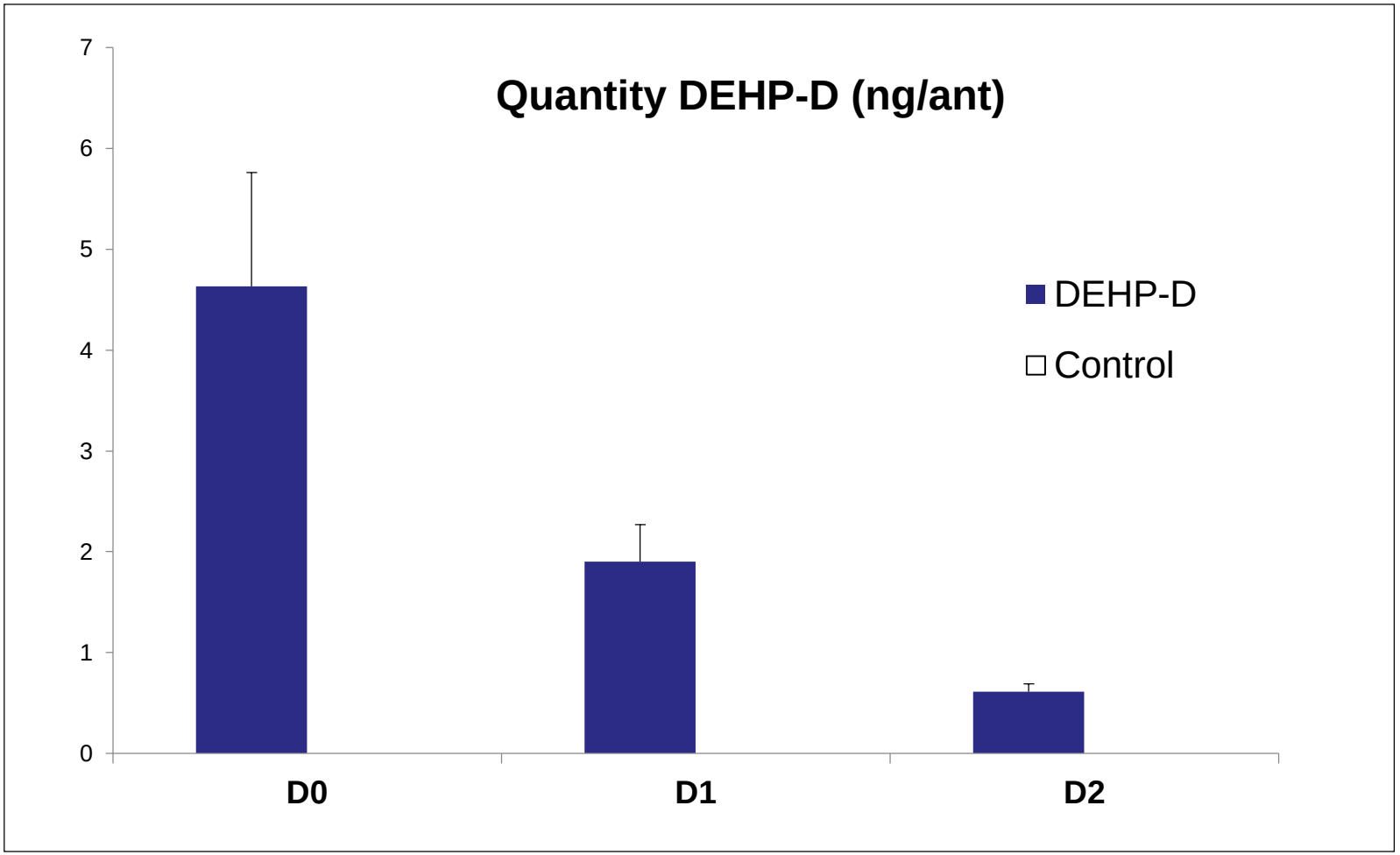
Deposited onto the cuticle, phthalates disapear with time



Lenoir et al 2014

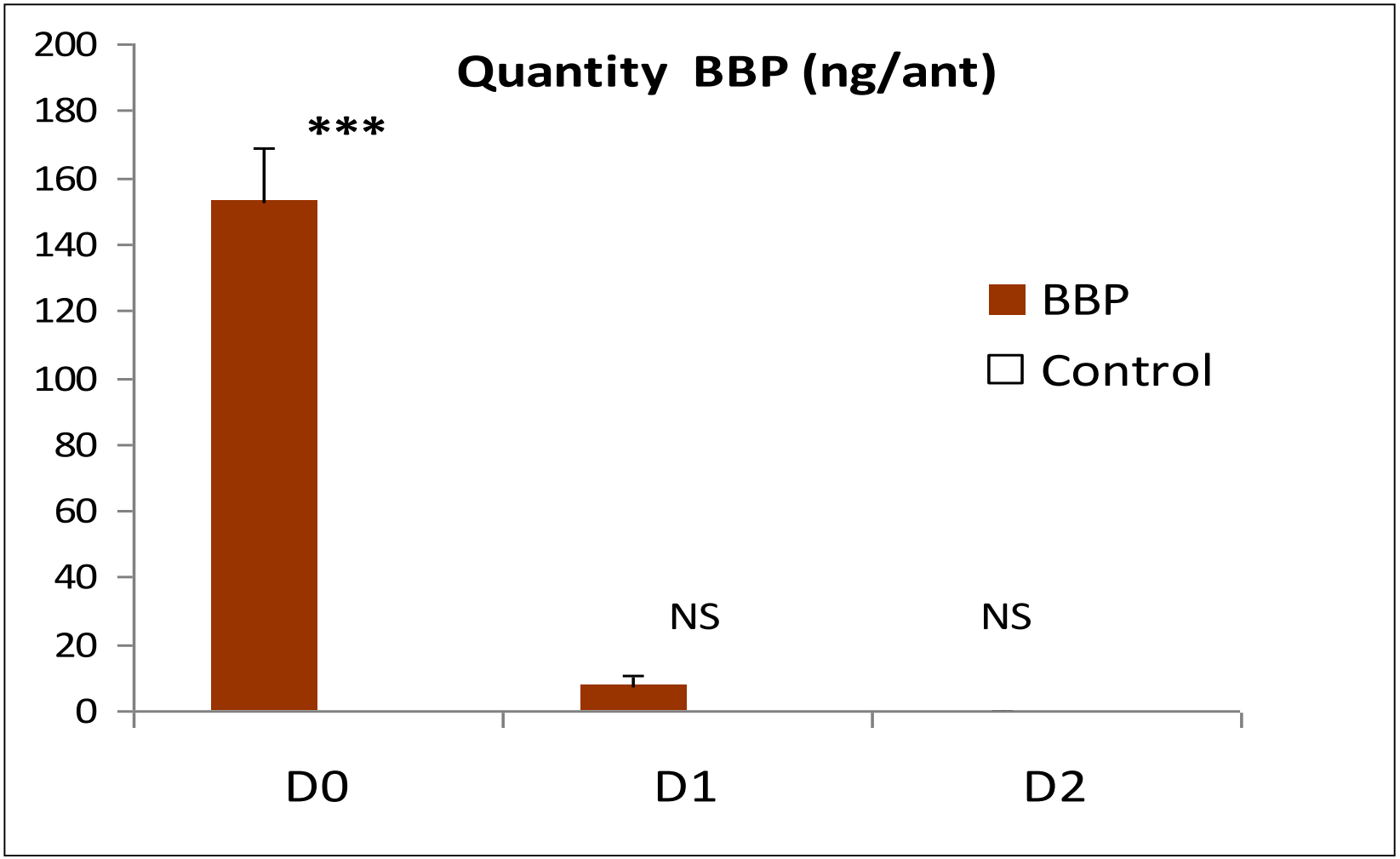
Same phenomenon with control components

Deuterium-labeled DEHP



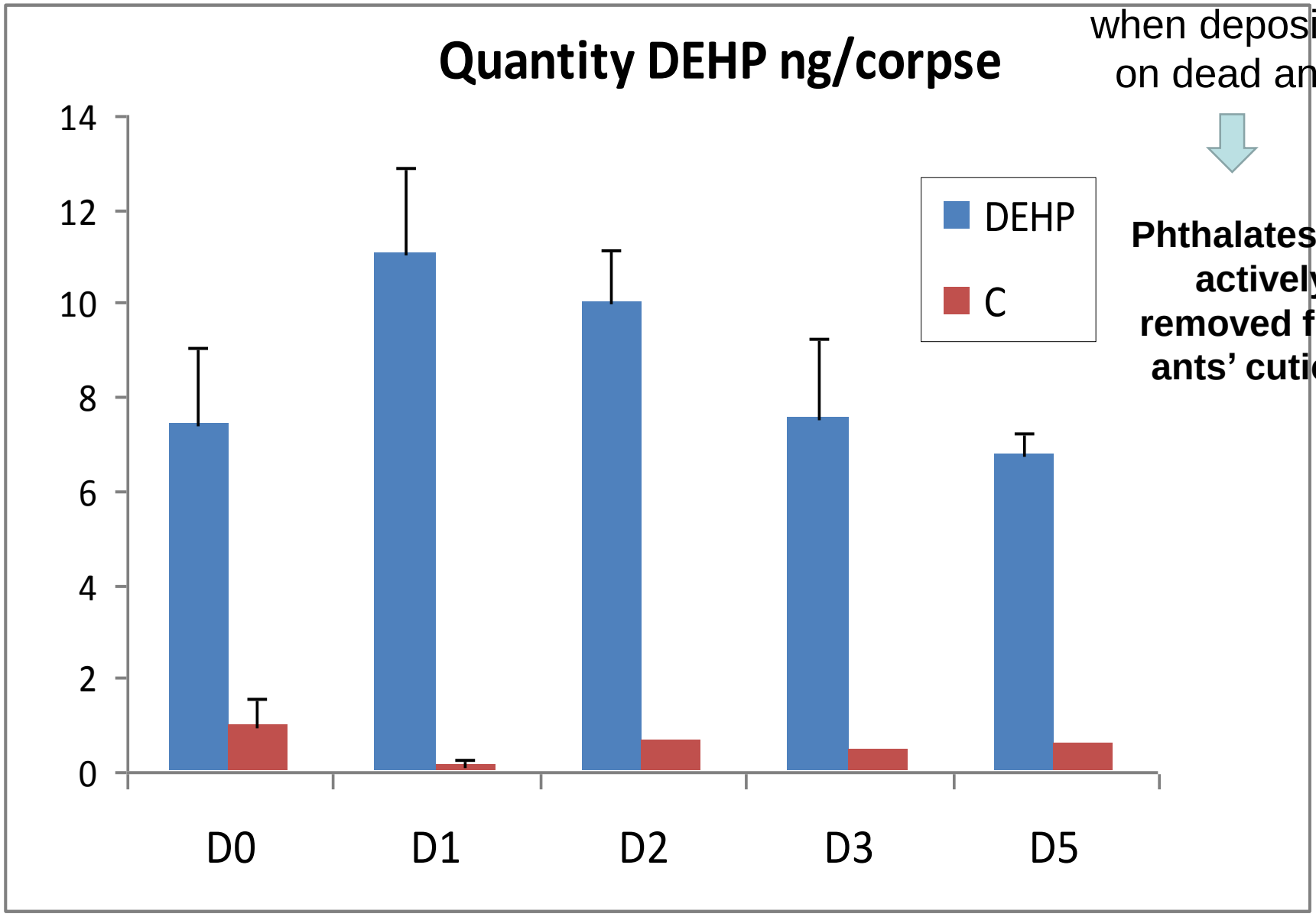
Lenoir et al 2014

BBP, phthalate never found on ants sampled in the wild in Touraine



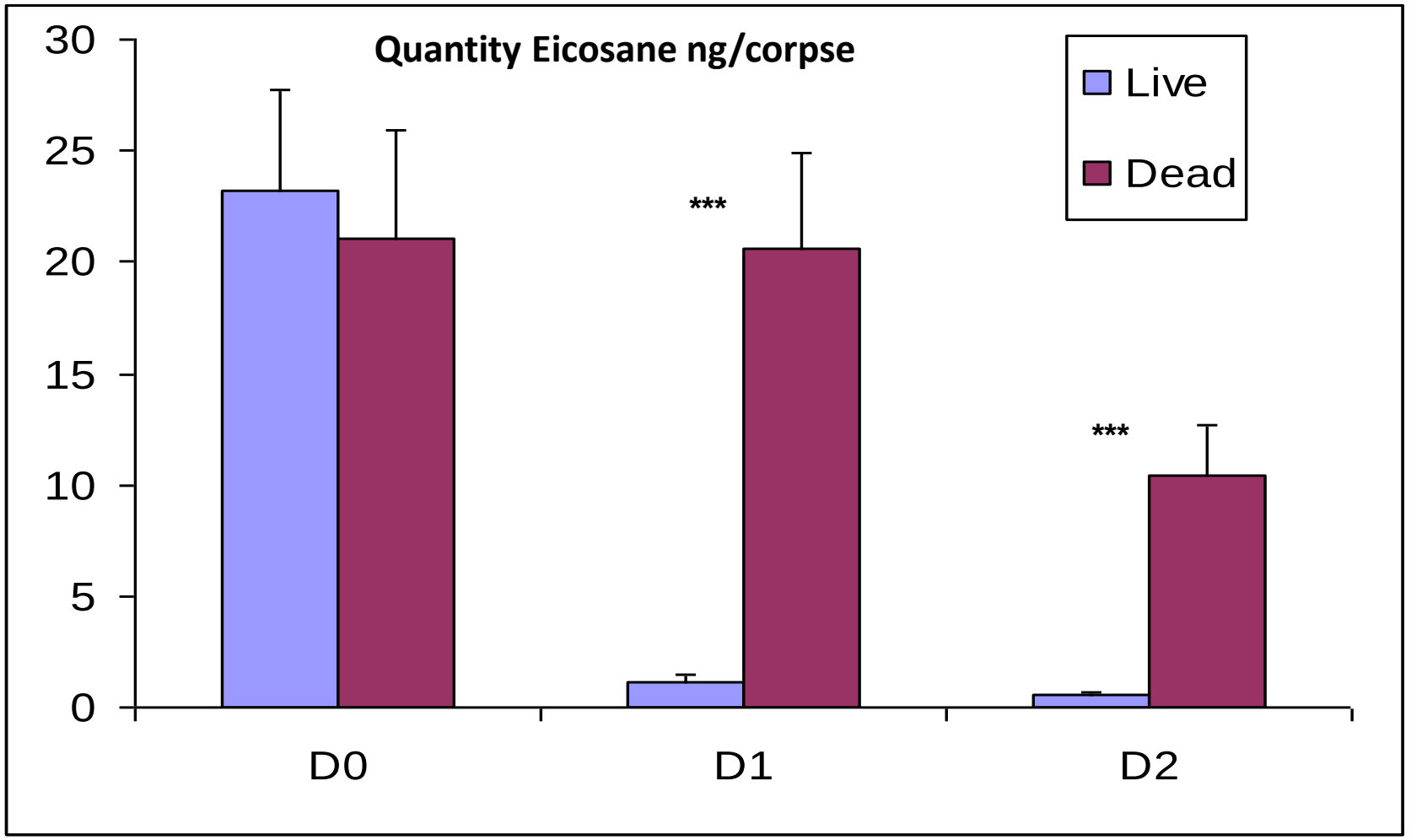
Lenoir et al 2014

DEHP do not disappear when deposited on dead ants



Phthalates are actively removed from ants' cuticle

Lenoir et al 2014



Same results with Eicosane (n-C20),
a control hydrocarbon
not found in the cuticular
signature of *Lasius niger*

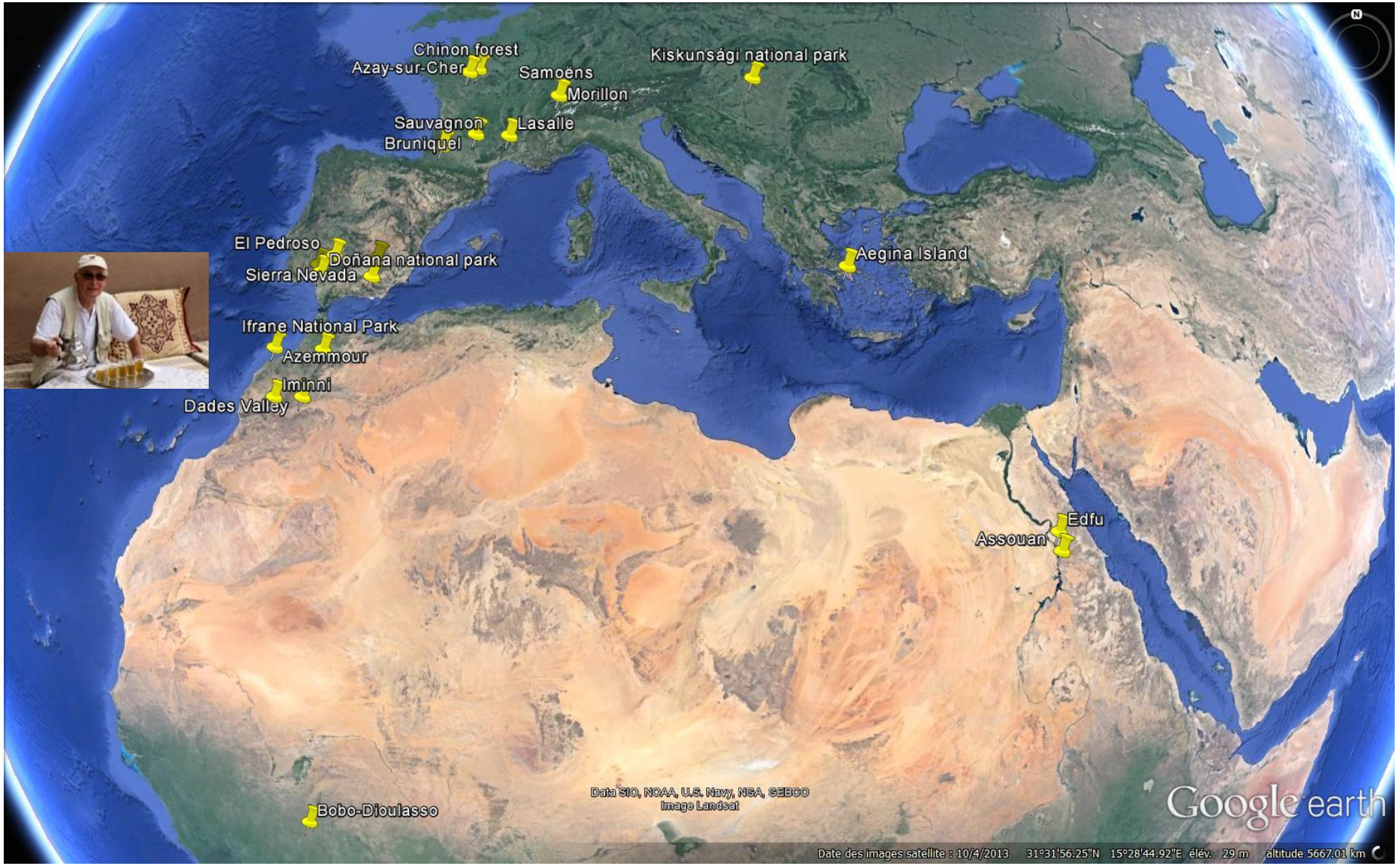
Lenoir et al 2014

Phthlates Who? Where?

- in all insect species studied, also honeybees
- everywhere without any direct contact with plastics
- into glands like PPG and Dufour
- on walls of the nests and on the foraging arena

- retrieved also using SPME

Contamination on all species tested, in all places sampled

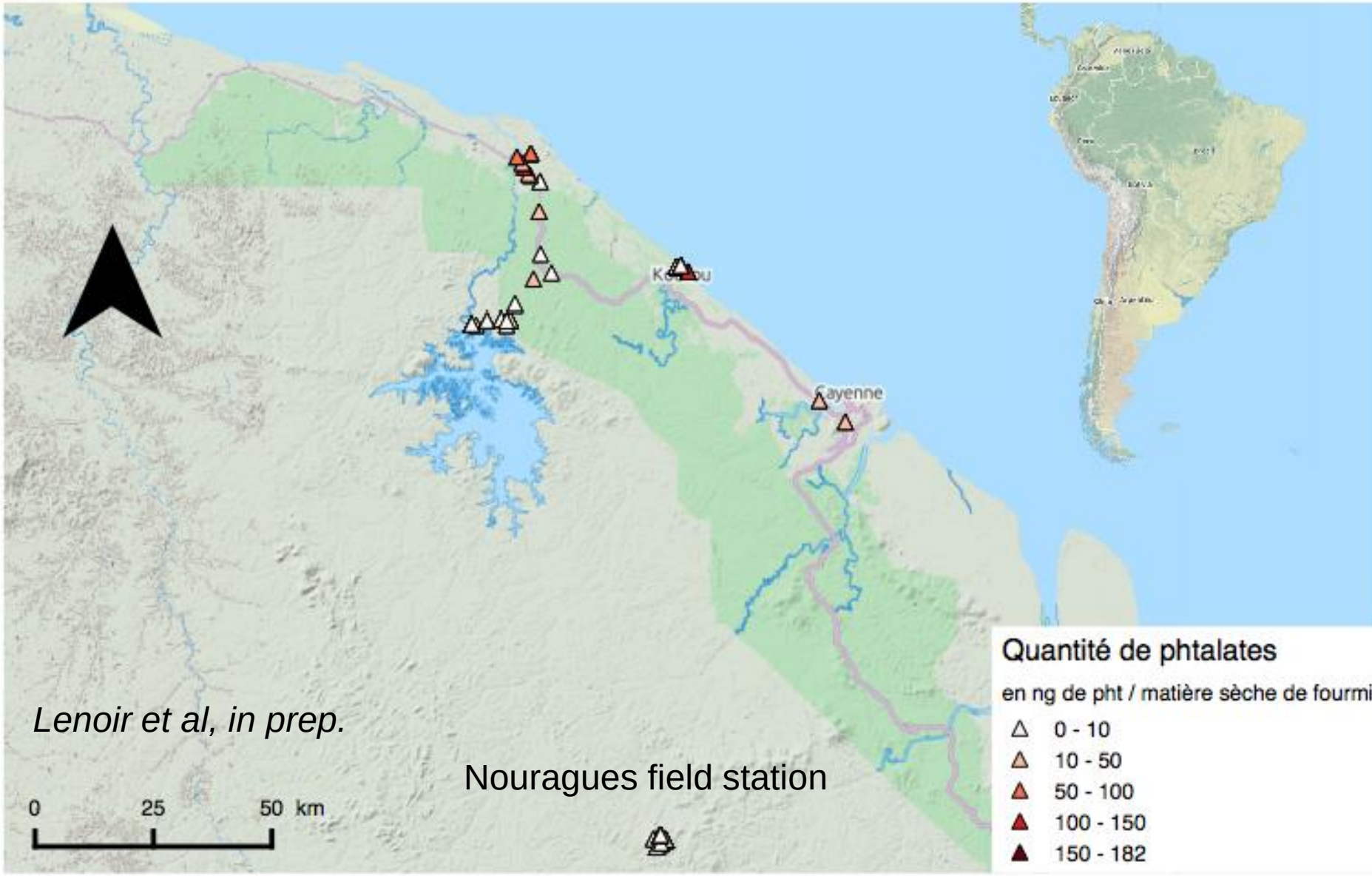


Lenoir et al. 2012 Science of the Total Environment

Guyana novembre 2013

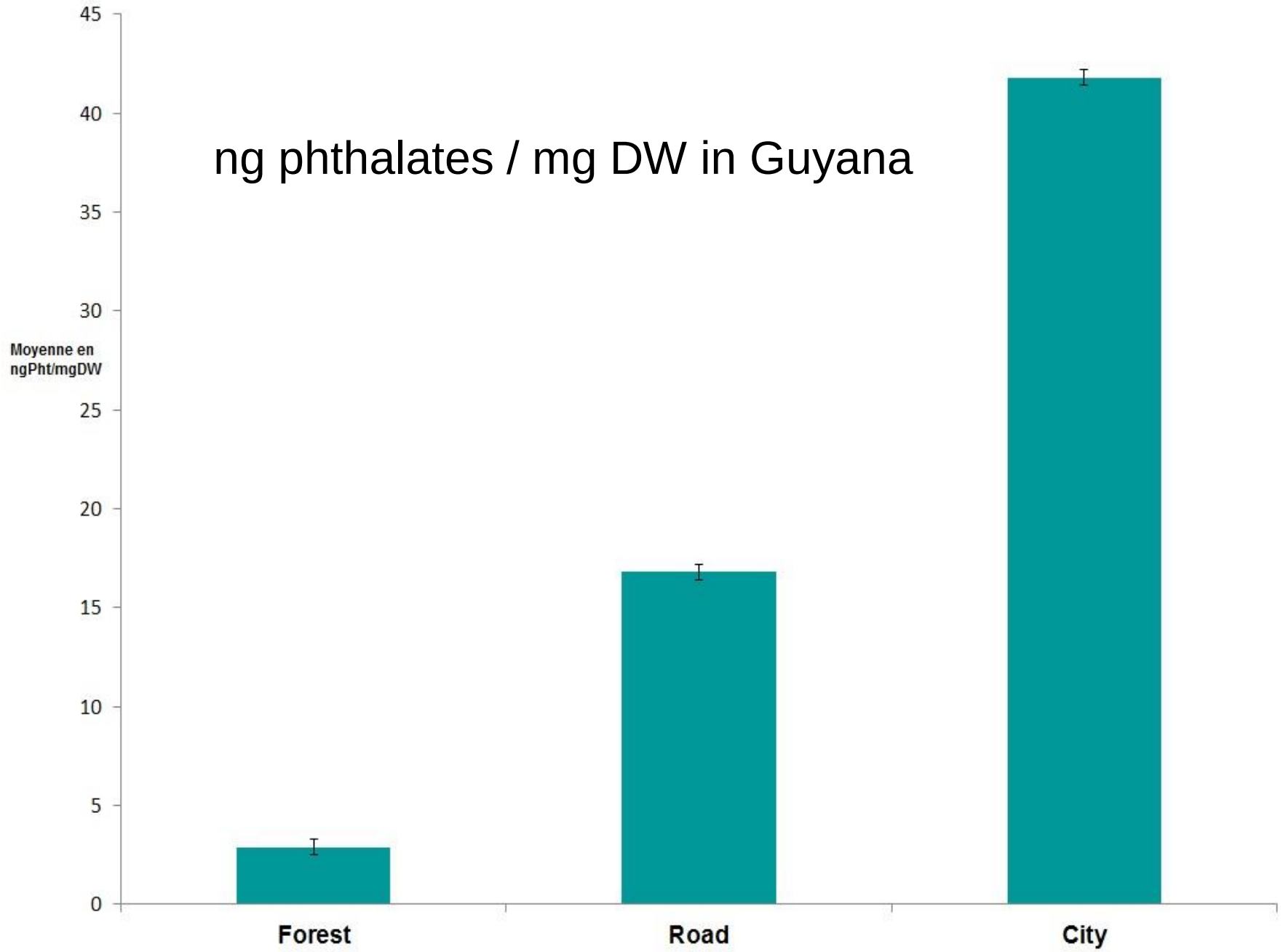
Nouragues Inselberg

Carte de la Guyane française



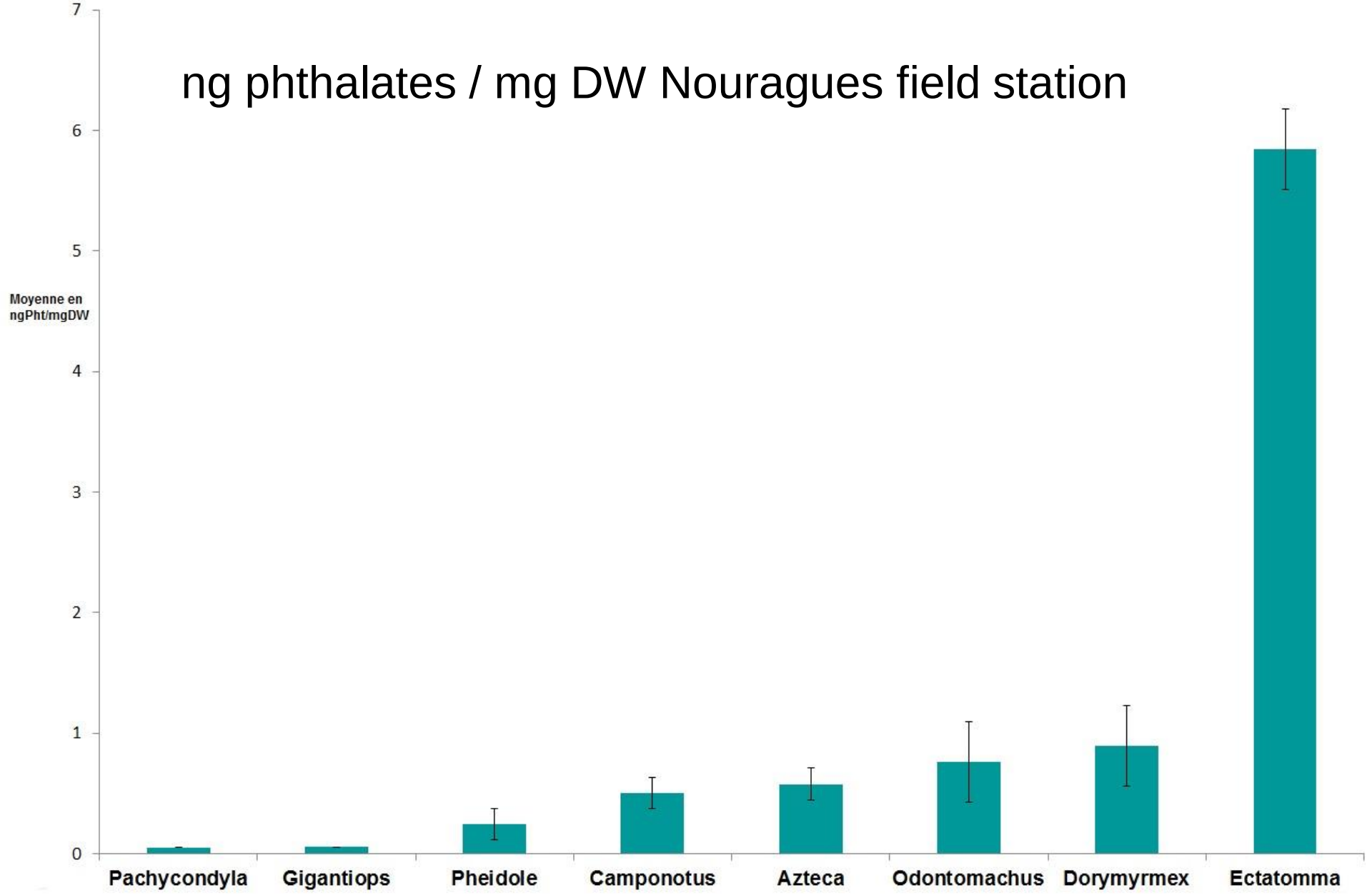
Lenoir et al, in prep.

Nouragues field station



Lenoir et al, in prep.

ng phthalates / mg DW Nouragues field station



Lenoir et al, in prep.

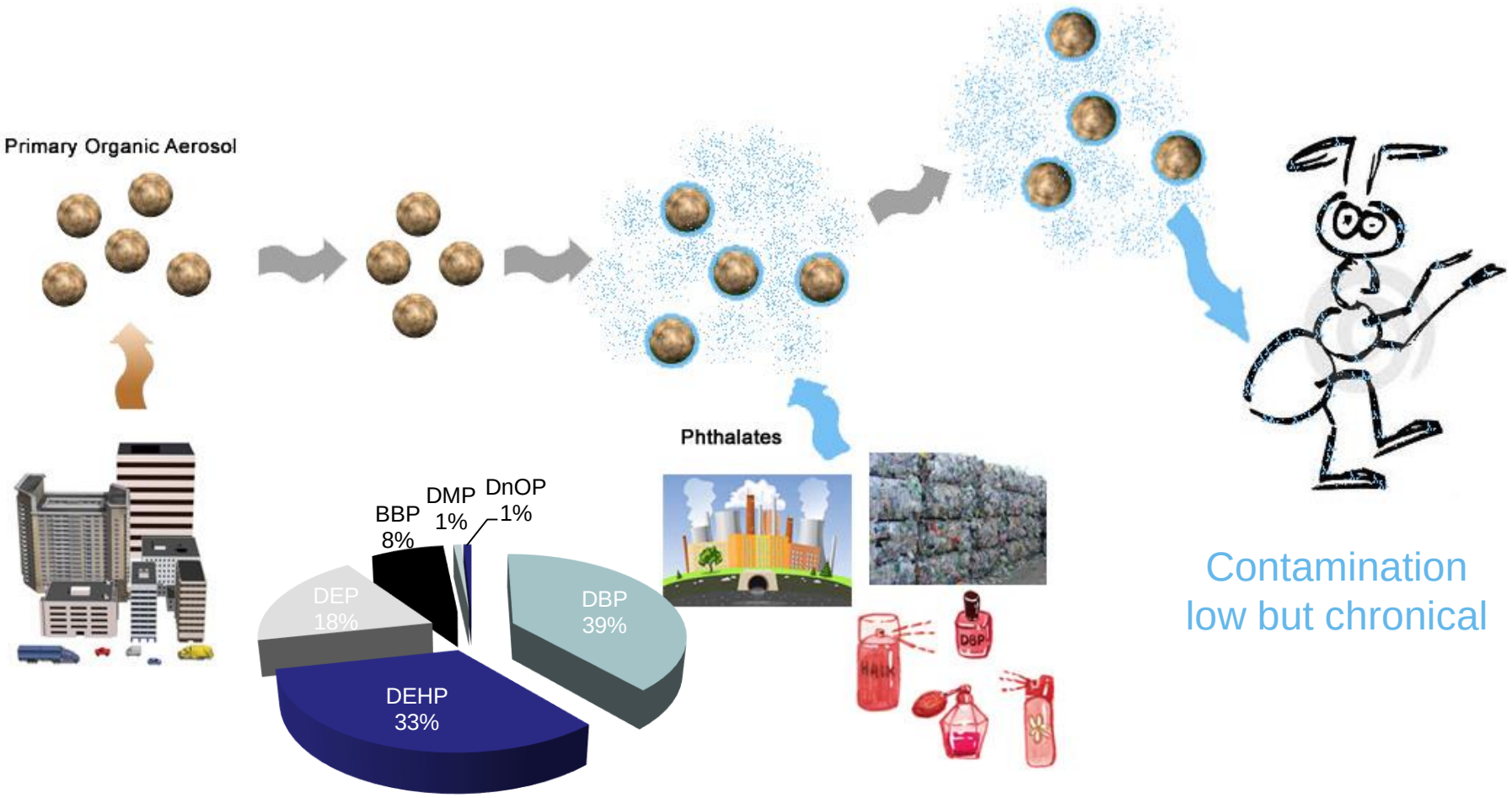
In the air?

Most of organic molecules spread in the air as aerosols

DEHP in dust: 100 à 7 000 microgr/ g

=> In the lab, in ant nests

Contamination by phthalates vaporized in the atmosphere or adsorbed on atmospheric particles

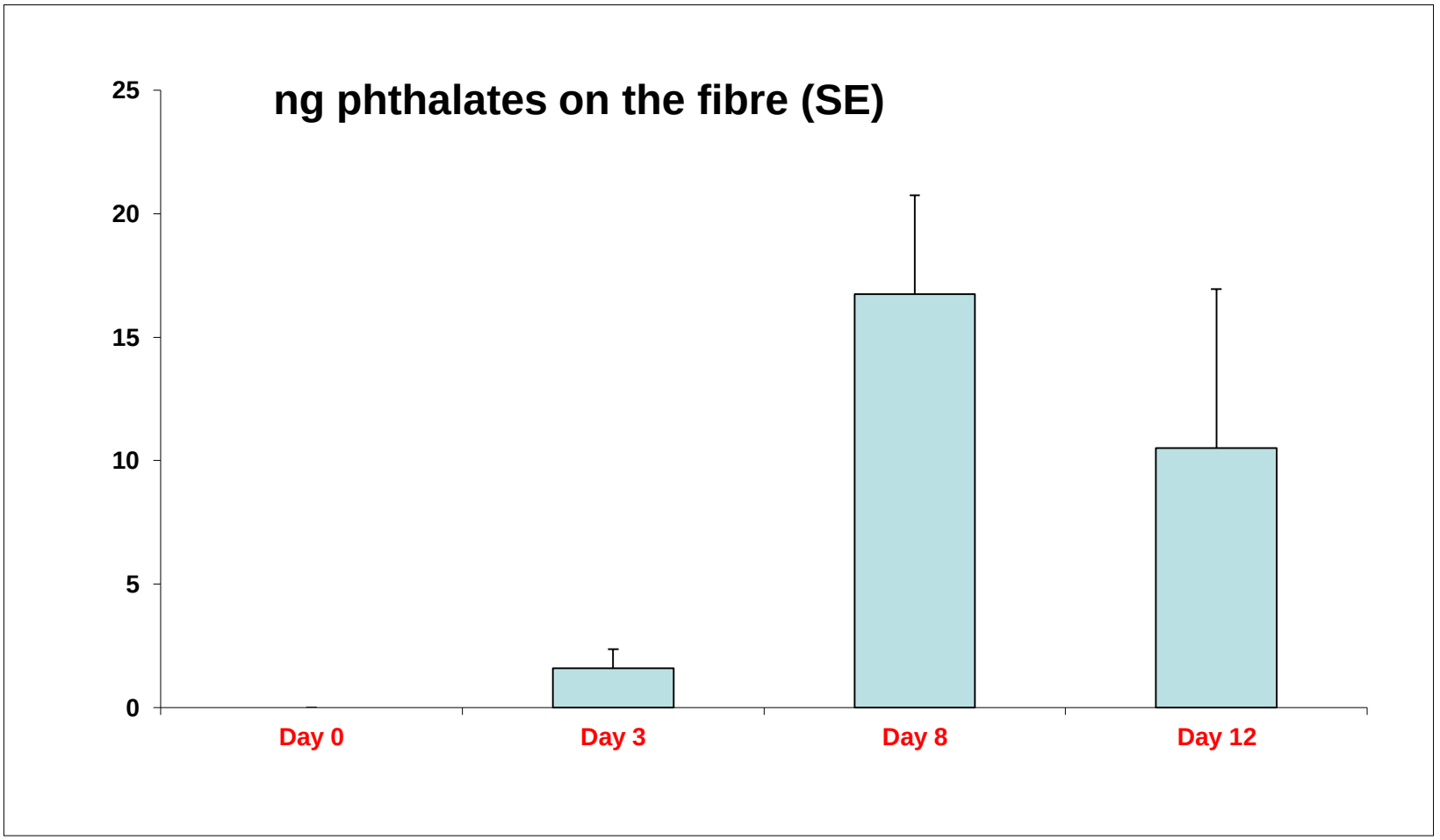


Main phthalates in Paris atmosphere (2002-2003)

(from Teil et al. 2006)

In the air?

Fibre SPME exposed to ambiant air



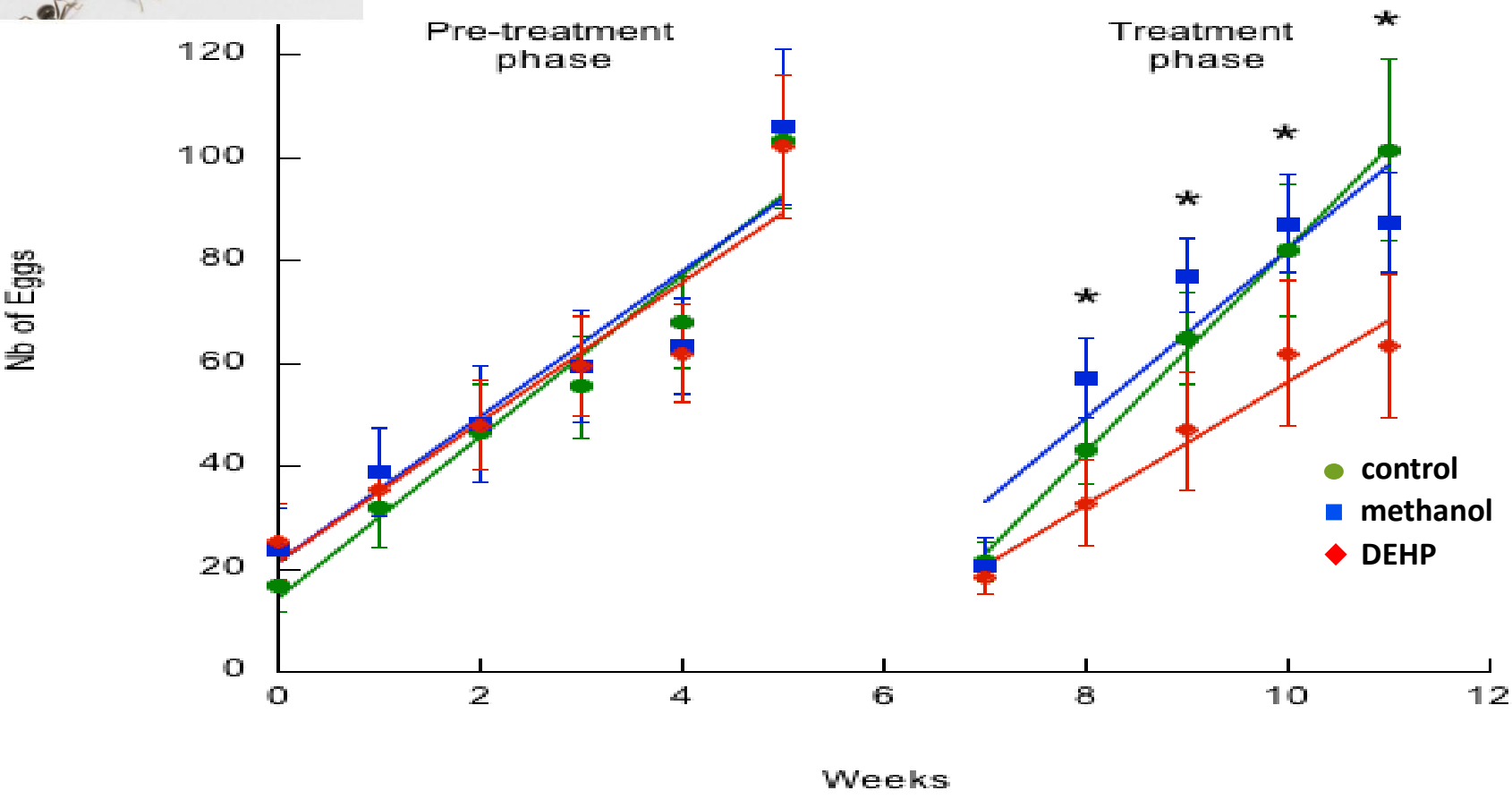
So, we know that…

- Ants are probably contaminated by atmospheric particules coated with phthalates, far from human activity
- Phthalates are adsorbed onto the cuticle of ants
- High phthalate doses are actively removed from the cuticle by the general cuticular dynamic
- Low doses remain chronically on ant's cuticle

? Physiological effect of such contamination ?

Effect on queen fertility?

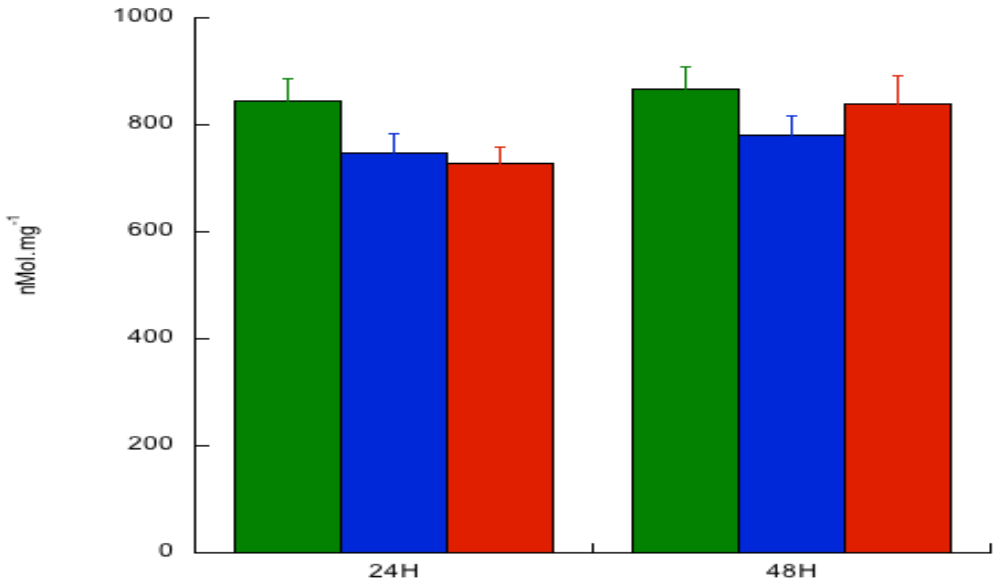
Lasius niger foundations



Queens exposed to DEHP lay fewer eggs

Cuvillier-Hot, …., Lenoir 2014

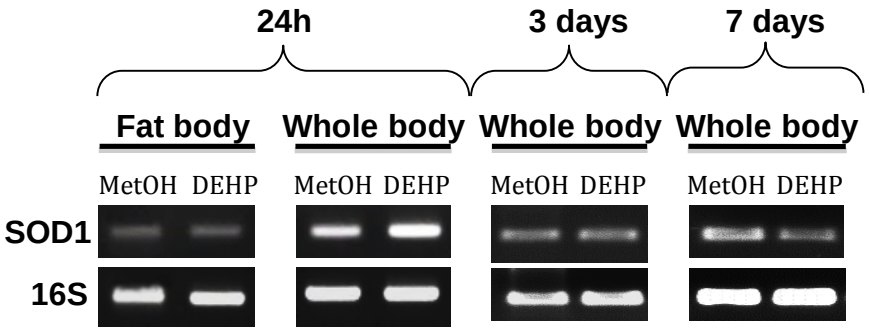
Effect on oxidative stress?



Malondialdehyde MDA

- control
- methanol
- DEHP

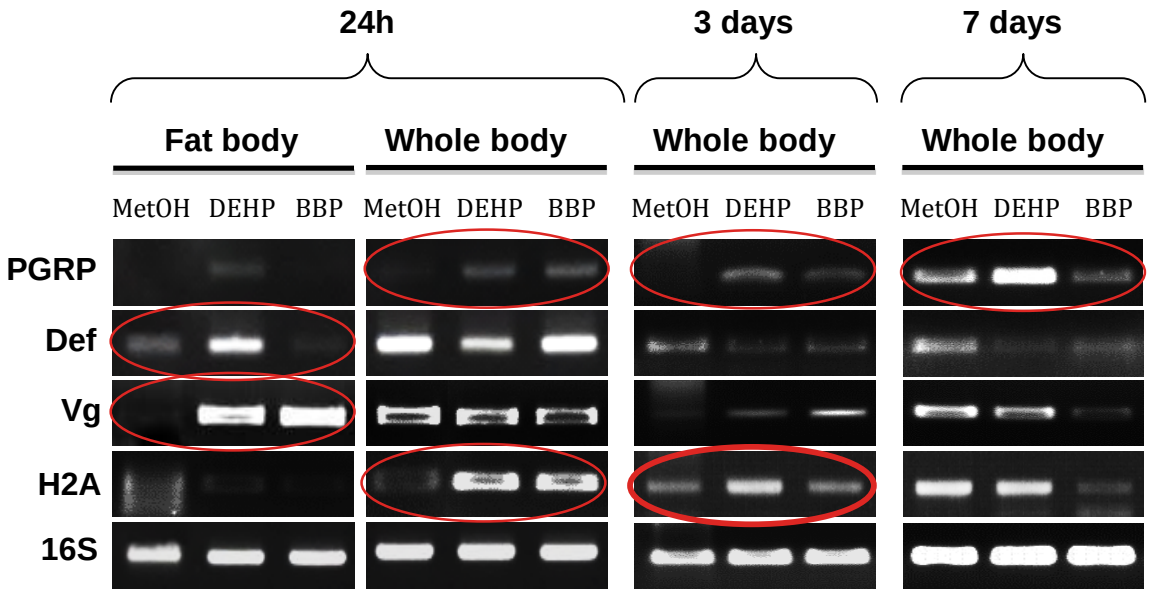
No oxidative stress induced by DEHP treatment after 24 or 48h



No induction of SOD1 expression

Cuvillier-Hot, …., Lenoir 2014

Detection of the chemical agression?



↑ 24h and 3d
↑↑ 7d — *PGRP* is a pattern recognition receptor that binds the peptidoglycans of bacterial cell walls

↑ 24h — *Defensin* is an antimicrobial peptide that disrupts the bacterial cytoplasmic membrane

↑↑ 24h, ↑ 3d — *Histone 2A* (H2A) may have an evolutionarily conserved role in innate immune defense

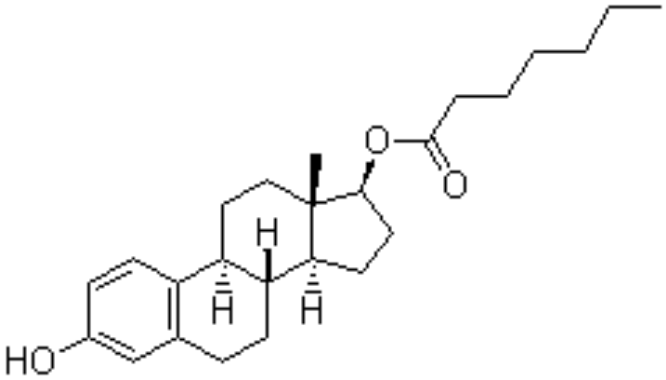
↑↑ 24h, ↑ 3d — *Vitellogenin* (Vg) also have important function in the immune system in social insects

Activation of the immune system

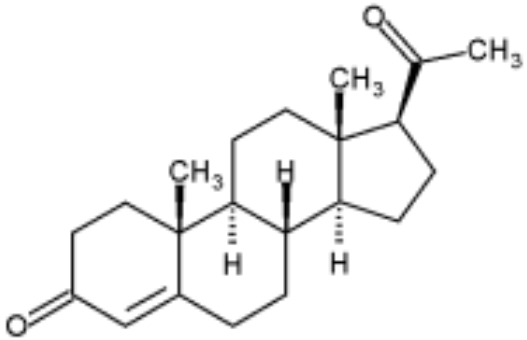
Cuvillier-Hot, …., Lenoir 2014

Action of phthalates

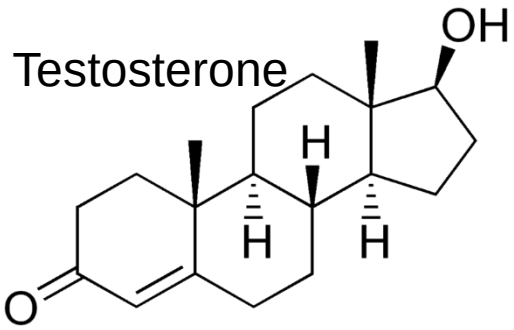
Aromatic ring like sexual hormones of vertebrates -> agonist or antagonist



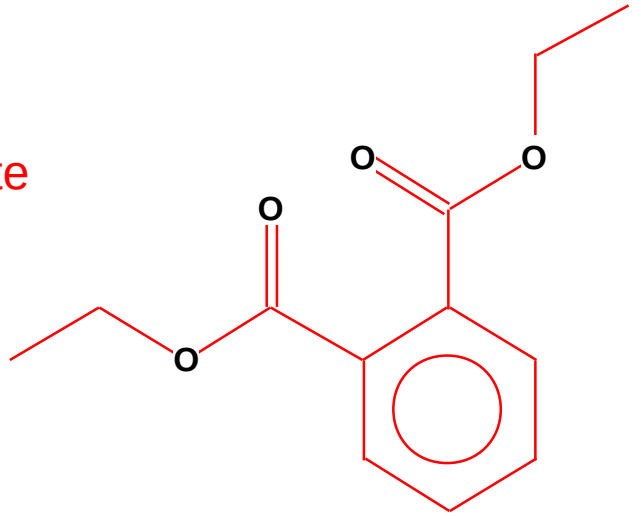
Oestradiol



Progesterone



phthalate



Phthalates: Universal contaminants

- In the 1990s: suspected to be involved in endocrine disruption because of anti-estrogenic effects

Main known effects:

- Disruption of normal development and reproduction, effects observed in one or more animal species (at least for DEHP, DBP and BBP) -> sterility
- toxicity to kidneys, on repeated exposure (workers, transfused or hemodialysed people)
- production of reactive oxygen species (ROS), cellular stress and oxidative damages (→ immunotoxicity)
- Link with obesity, insuline resistance, asthma, allergies?

EDCs: Endocrine Disrupting Components

EDCs since 50 years

- **Phtalates (everywhere) : plastics**
- Pesticides : atrazine, cypermetrine, DDT, chlordane, pyrethroids (different of natural pyrethrines), …
- Bisphenols : detergents, polycarbonate plastics (baby bottles, vials for micro-wave)
- PCBs (dioxines, construction materials, paintings, insecticides)
- parabens (antimicrobials): solar creams, jams, sirups
- contraceptive pills (oestrogens: ethinyl-oestradiol)

Now more than 600 substances

New phtalates

Ants with duck

Quantité DEHTP ng/Fi

Conclusion

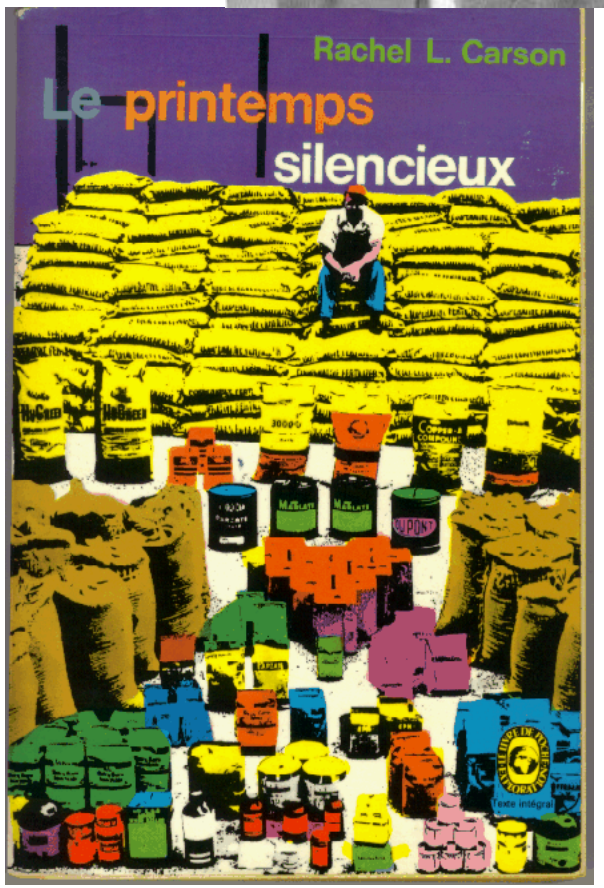
Major problem of the XXI century will be climate change but also pollution by EDCs (not considered in COP21)

= poisoning the humans

Ants good indicators


NICOLAS VIAL

Rachel Carson



1907 - 1964

Silent spring 1962: pesticides (DDT)
Birds and fire ants

DDT Forbidden USA 1972

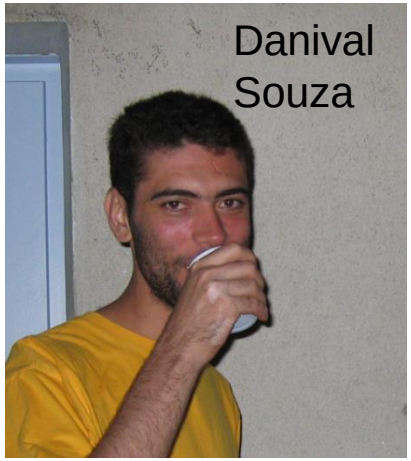
Theo Colborn (1927 - 2014)

EDC Endocrine Disruptive Chemicals

Wingspread declaration (1991)

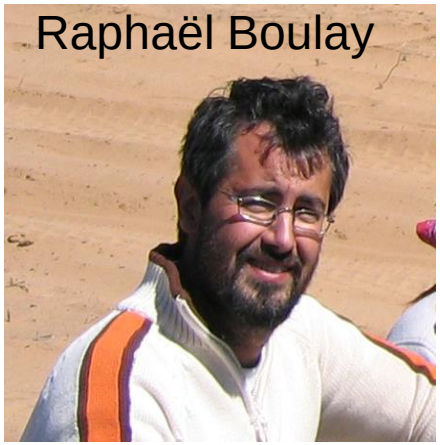
« Les héros de l'environnement »
special issue of Times 29 oct 2007

Theo Colborn
Chemist then PhD at 58 years
1980 discovers that females
of predators in great lakes of
USA are defeminizated


Danival
Souza


Ana Maria Matoso Viana


Patrizia d’Ettorre


Raphaël Boulay


Virginie
Cuvillier-Hot


Élise Nowbahari

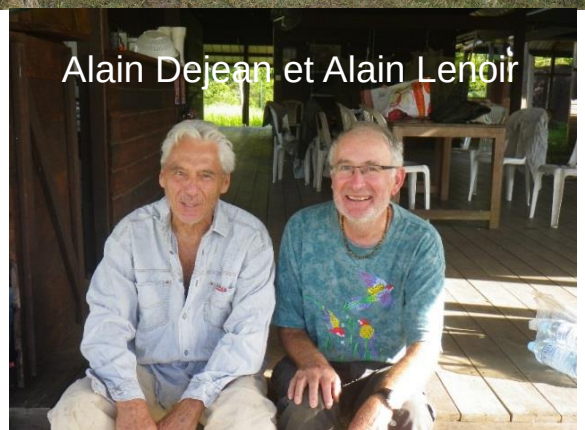

Katsuya Ichinose

Alberto Tinaut Paqui Ruano

Christine Errard

Abraham Hefetz

Xim Cerdá et Elena Angulo



Alain Dejean et Alain Lenoir