

Thesis project, will be submitted at:

PhD Program

University of Lyon, University Claude Bernard Lyon 1

PhD Application Form at E2M2

Academic Year 2018/2019

Research Unit at University of Lyon

Name of the Research Unit: **MAP Microbiologie, Adaptation, Pathogénie**

Identification code (N°EA, UMR.): **UMR5240**

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Supervising and mentoring during the PhD Thesis

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HDR holder = accreditation to supervise Research (year, name of the University): **2000, INRA**

Current position (Professor, Medical doctor professor, ...) or rank: **DR INRA**

Number of PhD Student currently supervised (except yourself): **1 potential 2019-2021 ANR (co-dir ED Matériaux)**

Detailed PhD Project

(with 3 to 5 scientific references)

Estimated date of the beginning of the PhD: **January 2019**

Field of research of the PhD*: (see the list at the end, only one choice is possible): **Paleoenvironments and evolution**

Title of the PhD project:

\$\$ Bacterial symbiosis and food source specialization in phyto-parasitic Hemiptera : a multi-time scale study

Microbiote et paléomicrobiote des pucerons: effets de taille et de niche alimentaire chez les hémiptères phyto-parasites, une étude à plusieurs échelles de temps

Summary of the PhD project:

Abstract:

E2M2 – ED n°341

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La taille des organismes est un élément important de leur valeur adaptative, généralement à corrélation positive dans un environnement de prédation, et plutôt négative pour les parasites. Dans certains contextes évolutifs, d'autres facteurs peuvent contrebalancer l'évolution vers de grandes tailles, par exemple le vol dans la transition dinosaures/oiseaux ou chez les insectes.

Le microbiote des animaux, c'est à dire l'ensemble des micro-organismes qu'il héberge, est un facteur écologique dont l'importance a récemment été spectaculairement réévaluée par les techniques d'identification par séquençage massif. Son influence sur l'évolution précoce des organismes (paleontologie) n'a cependant pas encore été bien évaluée pour diverses raisons techniques, et en tout cas jamais analysées dans le contexte des pressions de sélection sur la taille des organismes. Les insectes hémiptères piqueurs comme les pucerons sont un terrain idéal pour tester ces facteurs, car la pression "de niche" pour les petites tailles (spécialisation alimentaire sur les vaisseaux végétaux internes, c'est à dire parasitaire, à partir d'ancêtres à alimentation prédatrice) y est effective.

Nous nous proposons dans cette thèse, à partir d'une part d'experimentation sur pucerons et hémiptères actuels, d'autre part sur des séries fossiles de l'ambre libanais, de tester le lien entre taille de ces organismes et diversité bactérienne associée. Ces effets de taille et de niche n'ont à notre connaissance jamais été abordés et peuvent avoir eu des effets importants sur l'évolution de l'immunité de ces insectes. Ceux-ci étant des vecteurs importants des maladies des plantes (et d'autres insectes, de maladies animales et humaines majeures), l'identification de ces effets pourrait amener une contribution importante à la compréhension des mécanismes évolutifs de la vécution des maladies infectieuses. Des technologies de pointe seront mise en oeuvre au cours de cette recherche (séquençage d'identification bactérienne, microtomographie X des pièces buccales) ou développées (identification potentielle de traces bactériennes fossiles par spectrométrie de masse, microtomographie sur fossiles par SBFEM - microtomie électronique-).

Background:

Organism size is a synthetic character of organism life histories, and usually a positive driver of species competition within an evolutionary time and space frame. In other terms, according to life-history theory, growth rates are subject to strong directional selection due to reproductive and survival **advantages associated with large adult body size** [1]. Animals usually develop "*active resource encounter*" strategies [2], among which predatory *heterotrophy* is the prominent mode (*vs osmotrophy, phototrophy or mixotrophy* encountered in lower-sized eukaryotes and prokaryotes).

As such, insects have developed on *ancestral predatory heterotrophic feeding* strategy, with solid food incorporating environmental bacteria, resulting in a "standard" animal microbiota [3]. However, certain insect taxa developed specialized feeding [4], resulting in notable departure from this microbiological encounter paradigm. Hemiptera are actually recognized mainly by their piercing-sucking mouthpart structures [5, 6]. This evolution has two interacting effects on the bacterial relationships of the host insects: a **size** effect, and a **niche** effect. Size effect comprise all pressures induces by feeding specialization (adaptation to target plant tissue); Niche effect comprise the pressures related to the passage from an open-microbe to a closed-microbe (within plant) environment. These factors have been notoriously underexplored in the general evolutionary correlates of size [2], probably due to restriction of their effects at a boundary-scale of sizes (the "*around- μ* " cell-size scale, related to the **purely intracellular feeding** of many evolved hemipteran clades, a rare feature in metazoan/ animal evolution).

In term of evolution of microbial symbioses in *Hemiptera*, the main driver hypothesized to play a role is the trophic factor, namely the colonization of a poor or nutritionally-unbalanced food, driving an obligate association with an autotrophic micro-organism.

In this Ph.D., we propose to explore the two other factors, size and niche factors, in the evolution of the hemipteran microbiome at two evolutionary time scales: a **micro-evolutionary** scale within the *Aphididae*, including experimental approaches, and a **macro-evolutionary** scale at the whole *Paraneoptera* (hemipteroid assemblage), including meta-analyses and paleo-entomology approaches. To our knowledge, this link between size and immunity (said crudely), has never been tested in any animal group.

Namely, we predict a filter effect at the low-side of hemipteran size range (to be tested on aphids in the extant part of the present project)

Also predicted is a shift in the composition of the insect microbiota when comparing, by meta-analysis, the bacterial composition from ancestral / less specialized species (as thrips or certain heteropteran taxa) to more specialized species within the vessel-feeding Sternorrhyncha for example; the former should be more diverse and generalist, while the latter should be less diverse more populated with plant-restricted bacteria.

Subject:

Research question: Decipher a possible link between feeding specialization (mainly through morphological evolution) and bacterial interactions in the Hemiptera taxon; evolution from parenchyma feeding (predatory feeding, analogous to necrotrophy) to vascular tissue feeding (parasitic feeding, analogous to biotrophy):

- Influence of organism size and feeding-part opening on the diversity and density of associated bacterial microbiota.
- Influence of mouth-part specialization and food regimen on the phylogenetic origin, diversity and density of the residing bacterial microbiota.

The project inserts into a wide **eco-evo context**, and should give rise to solid publications in generalist journals, especially when dealing with the macroevolutionary questions. Evolution of size regularly gives rise to high impact papers (such as the recurrent interest in multimodal size evolution in dinosaurs [7]), and a case linking size evolution and immunity should represent an interesting paradigm. It may be noted that our Lebanese collaborator (Prof. D. Azar), and his outstanding link to the MNHN group, has an excellent publication record (Nature 2013; Sci.Rep.2015 & 2016, Geobios 2018 :-); <http://publicationslist.org/azar>.

Methodology:

Extant (micro-evolutionary) approaches: mainly applied on *Aphididae*

- **A: infraspecific level** (focus / article)

A.1. Case-study of an exceptional size-plasticity (1000%) linked to host-adaptation in *Aphis gossypii*: the so-called yellow-dwarf phenotype [8]. A drastic size reduction has been observed in this species upon host-switch in several host-adapted races/genotypes, with no major impact on reproduction: aphids are more than ten times smaller after host-switch, and the adaptive value of this trait is not clear. We propose to explore the effect of this switch on this aphid's microbiota, with the expected effect that any horizontal transmission of microbiota should be drastically reduced on the dwarf phenotype, due to size effect (through lowering to zero of the mouth-uptake of bacteria in the dwarf aphids).

Two genotypes of *A. gossypii* will be used: 1 NM1 (cucurbit race, on melon), and 1 Burk (Malvaceae race, reared on cotton).

Rearing in semi-natural conditions (tunnel greenhouses at INRA Avignon), with host switch: NM1 on melon (H) and on cotton (NH), Burk on cotton (H) and on melon (NH). Comparative 2-way Anova analysis of the aphid metagenome/microbiota (16S-based). All analyses are feasible with standard published procedures, and will be executed in collaboration with INRA Avignon (N. Boissot, with eventual input from F van Leerberghe, CBGP INRA CNRS)[9].

A.2. Case-study of host-race size polymorphism (weak, more standard polymorphism) in the pea aphid *A. pisum*

Three host races with maximal size polymorphism will be selected (B, M, S; Big Medium Small) in *A. pisum* [10, 11], and sampling will also be operated on immature stages (small-sized) to confirm at field level previous *in vitro* observations,

mentioning a link between individual size and intestinal cultivable microbiota [12]. Field experiments on a single common host (*Vicia faba*) will be designed to lower confounding factors to a minimum. 2/3-way Anova to extract host-race (genetic) factor from the size factor.

- **B: Inter-specific level** (focus / article)

Re-analysis of existing bacterial metagenomic surveys in aphids (INRA SPE project JC Simon, to link with), and linking with species size as extracted from the taxonomic database “aphid species file” (Colin Favret; <http://aphid.speciesfile.org>).

Re-experimenting with a very restricted subset of taxa/species, chosen and optimized for experimenting on size effect (same plant species).

On such species: i) microscopy analysis stylet canal sizes, and measures of allometric/isometric links on organism and mouthpart sizes, and ii) limited bacterial metagenomic additional screens

Enlarging the study, by bibliographic meta-analysis to all Hemiptera / Paraneoptera, which share a potential common mouthpart origin.

Eventually enlarge to a small number of external taxa with similar global features, such as nematodes (same gradient of niche effect, from ancestral free-bacterial feeding to evolved phytoparasites), which also comprise stylet feeders.

Fossil / ancient (macro-evolutionary) approaches:

- **C: Fine evolution of the piecing-sucking apparatus:** links with target tissue and insect size.

Analysis of mouthpart of Hemiptera & Thysanoptera fossils in ambers and impression collections, and inference of potential plant taxa associated with these insects (food plants).

Use of advanced micromorphological techniques (X-ray synchrotron tomography and/or Micro-CT Scan) to visualize and reconstruct the mouthparts of key fossils within the collection.

Establishing cladistic phylogenetic reconstructions within the studied lineage and using mouthparts' characters data.

Calibrate the age of appearance of the main modern aphid groups from existing fossils, and establish models and evolutionary scenarii including available phylogenetic data on modern aphid symbiotic partners.

Test-try molecular geochemical characterization of microbial sources in a reduced sample of insect fossils, to characterize microbial paleo-activity. The first trial would be that of bacterial lipids.

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Signatures _____LYON, (date) April 10 2018

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