PhD School on Agriculture, Environment and Bioenergy

(http://sites.unimi.it/dottorato_aab/)

(XXXIV cycle, 2018-20)

Project draft

1.Field of interest

General and applied entomology, Plant pathology (AGR11, AGR12)

2.Project title

Multilevel interactions between plants, microbes and insects: ecological and evolutionary constraints underlying interactions

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4.Relevance of the topic and state of the art:

Plants, microbes and insects are linked in complex networks of interactions, in which the organisms reciprocally influence each other leading to a continuous co-evolutionary arms-races, driven by the evolution of strategies to overwhelm the counterpart defense barriers (e.g., Pennacchio and Strand, 2006; Lorito 2010). However, we still have a limited view of the functional and ecological aspects of complex multitrophic interactions affecting the capability of insects of exploit plant tissues and trigger plant defense barriers (Sugio et al., 2015; Hacquard et al., 2017). Previous studies have focused mainly on the plant's chemistry and/or on the performance of the insects, but for the current proposal a third layer in the food-web (i.e., the microbiota) may be critical in determining the characteristics and evolutionary dynamics of plant-insects interaction. This hypothesis has recently taken in account, as in the case of the coffee berry borer (Ceja-Navarro et al., 2015), in the tortoise beetle *Cassida rubiginosa* (Salem et al., 2017) and in other leaf beetles species (Montagna et al., 2015).
Leaf Beatles (Coleoptera: Chrysomelidae), including numerous species considered pests of crops (e.g. *Leptinotarsa decemlineata*, *Oulema melanopus*, *Diabrotica virgifera*), represent a suitable and unique model to test for the existence of patterns in the structure of the insects associated microbiota due to the insects phylogeny or due to others ecological factors, such as the insect trophic guilds and the presence of secondary metabolism in tissues of the exploited host plants. Unravelling and understanding the mechanisms underlying these complex multitrophic interactions can help the development of bioinspired strategies for a sustainable crop protection.

5.Layout of the project

5.1.Materials & Methods:

A wealth of technologies, from high-throughput sequencing and behavioral assays to comparative tools and microscopy, associated with the interdisciplinary expertise of the research team, spanning across entomology, plant pathology and physiology, and molecular biology, will be used to disentangle insects-microbes-plant interactions.
The adopted strategies are addressed to: 1) characterize the microbiota (gut microbial community and p- and s-endosymbionts) associated to ~500 leaf beeatles species; 2)

integrate the insect host plant information from bibliography with molecular characterization of the insect gut content; 3) obtain a multi locus-based phylogenetic tree of the analysed species; and, 4) adopt comparative methods to test the hypotheses of the project.
The insects microbiota will be characterized using culture-independent methods targeting different regions of the bacterial 16S rRNA; amplicon sequencing will be performed using Illumina or IonTorrent technologies able to obtain sequences of suitable length for cluster analyses and taxonomic identification; dedicated pipeline analyses working under the QIIME platform will be developed and optimized. The characterization of the insect diet starting from DNA of the gut content will be performed using the metabarcoding approach targeting plant markers mostly used for this purpose (i.e. *matk* and *rbcL*).
Moreover, for some dominant symbionts the intimate relationship with their insect will be investigated by means of electron microscopy and of fluorescent in situ hybridization assays.
Besides the microbiota taxonomic characterization we aim to: 1) characterize the metabolic potential of the identified microbiotas; 2) correlate this potential with characteristics of the host plant and 3) test the hypothesis that insects feeding on the same host plant share common features both in term of bacterial taxonomy and in their dominant symbiotic metabolic pathways. The microbial metabolic potential associated with the various species and sources of insects will be predicted by using PICRUSt and the phenotype-based functions: BugBase and FAPROTAX.

5.2.Schedule and major steps (3 years):

First year: planning of the research activities, bibliographic research, collecting samples and mining sequence data (with the associated metadata) from previously published studies focused on insect microbiota.
Second year: identify plant metabolites, present in the exploited host plant, possibly interacting with the insect physiology, benefitting from the host plant databases of European leaf beetles already available in our research group and of the PCIDB - PhytoChemical Interactions DB. Extract DNA from samples, profiling of the bacterial community associated with ~500 species of leaf beetles, analyses of the data in order to test for the presence of ecological/nutritional (e.g., presence of toxic plant secondary metabolites) or phylogenetic constraints in shaping the associated microbiota. This part of analyses should be performed abroad benefitting of the already existing research collaborations. Gut content characterization using DNA metabarcoding approach.
Third year: writing manuscripts and presentation of the achieved results in national and international congresses.

6. Available funds

Matteo Montagna: 23354 CTE INT18MMONT 01 (12.000,00 euro)
Franco Faoro: CTE NAZPR 17 (4500,00 euro)

6. Literature: max 10 citazioni

Ceja-Navarro JA et al (2015). Gut microbiota mediate caffeine detoxification in the primary insect pest of coffee. Nat Commun. 6: 7618.
Hacquard S, et al (2017). Annu Rev Phytopathol, 55: 565-589.
Lorito M, et al (1998). PNAS, 95(14):7860-7865.
Montagna M et al. (2015) Metamicrobiomics in herbivore beetles of the genus Cryptocephalus (Chrysomelidae): toward the understanding of ecological determinants in insect symbiosis. Insect Sci. 22: 340-352.
Pennacchio F, Strand MR (2006). Annu Rev Entomol, 51:233-58.

Salem H, et al M. (2017). Drastic Genome Reduction in an Herbivore's Pectinolytic Symbiont. Cell. 171: 1520-1531.
Sugio A, et al (2015). J Exp Bot, 66: 467-478.