PhD School on Agriculture, Environment and Bioenergy

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(XXXVII cycle, 2021-24)

Project draft

1.Field of interest

General and applied entomology (AGR-11) and General Botany (BIO-01)

2.Project title

Microbiota of phytophagous insects: ecological and evolutionary drivers

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

Insects and microbes are linked in complex networks of interactions, where organisms reciprocally influence each other (e.g., Moran 2006), resulting in some cases in the expansion of the host niche space (e.g., see Lemoine et al. 2020). Concerning the insect-plant interactions, we still have a limited view of the functional and ecological aspects affecting the capability of phytophagous insects to both trigger plant defense barriers and exploit plant tissues (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 recent discoveries highlight the existence of a third layer in the food-web (i.e., the microbiota) that may be critical in determining the characteristics and evolutionary dynamics of the interactions between phytophagous insects and their host plants (e.g., Ceja-Navarro et al., 2015; Montagna et al., 2015; Salem et al., 2017). Nowadays, little is known about the ecological and evolutionary factors that contribute shaping the insect microbiota structure and functions. Some studies recognized trophic guild as the main driver (e.g., Colman et al. 2012), while others assigned this role to the insect environmental habitat (e.g., Yun et al., 2014). However, little attention has been dedicated to address these questions into an evolutionary perspective (i.e., are closely related insect species characterized by similar microbiota?).
Leaf beetles (Coleoptera: Chrysomelidae), including numerous species considered pests of crops (e.g., *Leptinotarsa decemlineata*, *Oulema melanopus*, *Diabrotica virgifera*), represent a suitable model to test if phylogenetic and/or ecological factors contribute to shape insects microbiota.
Unravelling and understanding main drivers of these complex multitrophic interactions can also 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 comparative tools, associated with the interdisciplinary expertise of the research team, spanning across entomology, botany and molecular biology, will be used to disentangle the microbiota associated with phytophagous insects with an evolutionary perspective.
The adopted strategies are addressed to: 1) characterize the microbiota (gut microbial community and p- and s-endosymbionts) associated to ~300 West Palearctic leaf beetles 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 useful to reconstruct the ancestral microbiota and test the influence of the phylogeny on its evolution; and 4) adopt comparative methods to test the hypotheses of the project.

Insect 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 2 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 relevant and not yet characterized insect-symbiont associations (detected after the 16S rRNA metabarcoding analyses), their intimate relationships will be investigated by means of shotgun sequencing coupled with a the so called "blobology" approach (Kumar et al., 2013). 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; 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; 4) test the hypothesis that polyphagous insects harbor a more diversified microbiota than monophagous insects; and 5) evaluate the impact of the phylogenetic relatedness of the analysed insect species on the microbiota structure and composition. 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 (including bibliographic research), mining sequence data (with the associated metadata) from previously published studies focused on insect microbiota. Extract DNA from ~500 species (three to five population per species) of Chrysomelidae already collected within 10-years surveys across the Mediterranean Basin and send it out for NGS sequencing.
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 Data Base. Bioinformatic analyses of the obtained 16S rRNA reads and statistical analyses on the obtained ASV table to test the project hypotheses. Shotgun sequencing insect-symbiont association of interest.
Third year: bioinformatic analyses of the obtained shotgun reads in order to assemble and annotate the symbiont's genome, comparative and predictive functional analyses in order to dissect the insect-symbiont relationship. Writing manuscripts and presentation of the achieved results in national and international congresses.

6. Available funds (source and amount)

Matteo Montagna: PRIN201719MMONT_01 (~5k euro)
Alberto Spada: PRIN201719FFAOR_01 (~10k euro)

6. Literature:

Ceja-Navarro, J.A., et al (2015). Nat Commun. 6: 7618.
Colman, D.R., et al. (2012). Mol ecol, 21: 5124-5137.
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Lemoine, M.M., et al. (2020). Curr Opin Insect Sci, 39:14-20.
Montagna, M., et al. (2015) Insect Sci, 22: 340-352.
Moran, N.A. (2006) Symbiosis. Current Biology, 16: R866-R871.
Salem, H., et al. (2017). Cell, 171: 1520-1531.
Sugio A., et al. (2015). J Exp Bot, 66: 467-478.