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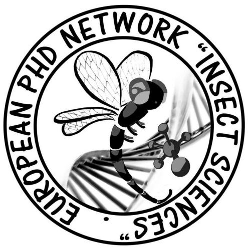

Scientific Program

Tuesday 6th November

14:00-15.45	**Registration at the Ostello Salesiano Eporediese**
16:00-16:15	**Opening of the Meeting**
	Keynote lectures
16:15-17:00	**ANTONIO CARAPELLI** Focus on arthropod kinships: hexapods and crustaceans thought to be cousins but eventually realized they are brothers
17:00-17:45	**RODRIGO P.P. ALMEIDA** Basic science in an applied world: insights on the leafhopper transmission of a plant pathogen
18:00-19:00	**Welcome cocktail**
20:00-21:00	**Dinner**
21:00-22:00	**Meeting organization by PhD students**

Wednesday 7th November

Session 1. Physiology and Development

8:45-9:15 **Plenary talk**	**Klaus H. Hoffmann** Juvenile hormone titer and allatoregulating neuropeptides in wood-feeding cockroaches and primitive termites
9:15-9:45 **Plenary talk**	**Gianluca Tettamanti** Midgut remodeling in lepidopteran larvae: the contribution of autophagy and apoptosis
9:45-9:50 **Poster**	**Manuela Camerota** Hsp70: studies on expression/overexpression in vivo in *Drosophila melanogaster* during rapid cold hardening (RCH) and after exposure at low temperatures
9:50-9:55 **Poster**	**Marlène Chirault** Consequences of environmental stresses on male fertility, physiological and molecular aspects in the model *Nasonia vitripennis*
9:55-10:00 **Poster**	**Barbara L. Ingegno** Physiological and behavioural assays to explore host plant selection in two insect systems
10:00-10:15	**Laura Berardi** Genetic characterization of the urticating system in processionary moths of the genus *Thaumetopoea* (Thaumetopoeinae, Notodontidae)
10:15-10:30	**Sandy Weidlich** Regulation of digestive enzymes in the Mediterranean field cricket *Gryllus bimaculatus*
10:30-10:45	**Valentina Lasco** *Drosophila melanogaster* as model system to study an evolutionarily conserved gene involved in insect development and immunity
10:45-11:15	**Coffee Break**

Session 2. Insect behaviour	
11:15-11:45 **Plenary talk**	**VALERIO MAZZONI** Vibrational communication in insects: entangled and invisible networks on plants
11:45-12:00 **Plenary talk**	**MAJA DERLINK** Vibrational signals as a prezygotic reproductive barrier
12:00-12:15	**MARCO SALA** Acoustic behaviour in myrmecophilous insects
12:15-12:30	**ANKA KUHELJ** How stress affects the mating behaviour in the leafhopper species *Aphrodes makarovi*
12:30-12:45	**MABROUK BOUNEB** Olfactory response of a specialist and a generalist phytoseiid mite to cues emitted by tomato under combined infestation
12:45-13:00	**ANTOINE HUMEAU** The kinematic of ants walking on the sand and in the antlion trap
13:00-14:30	**Lunch**

Session 3. Insect interactions

14:30-15:00 **Plenary talk**	**Ilaria Negri** *Zyginidia* mRNA sequencing, where is the feminizing *Wolbachia* acting?
15:00-15:05 **Poster**	**Jérémy Gauthier** Genomic adaptation of the parasitoid *Cotesia sesamiae*
15:05-15:10 **Poster**	**Mahnaz Rashidi** Dissecting the effects of fusion phytoplasma membrane proteins on vector acquisition and transmission capabilities
15:10-15:15 **Poster**	**Elisabetta Viale** Phylogenetic relationships between European and Hawaiian species of the genus *Trupanea* (Diptera, Tephritidae) and their symbiotic bacteria
15:15-15:30	**Mélanie Body** Impact of leaf-miner insects on the primary metabolism of their host-plant: manipulating from the inside
15:30-15:45	**Elisabeth Huguet** Molecular basis of plant gall induction by cynipid wasps
15:45-16:00	**Germain Chevignon** First functional annotation of a polydnavirus: the expressed *Cotesia congregata* bracovirus explored
16:00-16:15	**Aurélien Chateigner** Ultra-deep sequencing of AcMNPV and comparison to original genome sequencing
16:15-16:30	**Luciana Galetto** Cytoskeleton proteins of the insect vector are involved in phytoplasma infection
16:30-16:45	**Monia Monti** Interactions between the phytoplasma ‘*Candidatus* Phytoplasma mali’ and the vector *Cacopsylla melanoneura* (Hemiptera, Psyllidae) in the transmission of Apple Proliferation in NW Italy
16:45-17:15	**Coffee break**
17:15-18:45	**Workshop “Beyond the lab: bringing science to the public”** **by Davide Ederle and Simona Palermo**

Thursday 8th November

Session 4. Insect control and biotechnology	
8:45-9:15 Plenary talk	**MARIANGELA COPPOLA** New insights on tomato defence responses
9:15-9:45 Plenary talk	**ELENA GONELLA** Insect-bacteria symbiosis: a potential tool for pest control
9:45-9:50 Poster	**FABIO MAZZETTO** Potential symbiotic control agents against *Drosophila suzukii*
9:50-9:55 Poster	**DIMITRIOS E. MILIORDOS** Development of new tools for the integrated management of Flavescence dorée of grapevine and the vector *Scaphoideus titanus*
9:55-10:00 Poster	**JULJA ERNST** Gallwasp community assembly across historical and regional scales
10:00-10:15	**AMBRA QUACCHIA** The natural enemy *Torymus sinensis* for the control of the Chestnut gallwasp
10:15-10:30	**ILARIA DI LELIO** Parasitoid-inspired selection of a target gene forRNAi
10:30-11:00	**Coffee break**
11:00-11:15	**HUGO MATHÉ-HUBERT** Causes of success and failure in biological control: venom contents and evolution in the parasitoid wasps *Psyttalia lounsburyi/P. concolor*
11:15-11:30	**JEAN-LUC GATTI** How much venom is necessary for the success of endoparasitoid wasps?
11:30-11:45	**AGOSTINO STRANGI** Evaluation of a cryopreservation protocol for*Galleria mellonella* eggs parasitized by T*richogramma brassicae*
11:45-12:00	**FRANCESCO BINAZZI** Life table of *Oencyrtus pityocampae* reared on *Nezara viridula* eggs

Session 4. Insect control and biotechnology	
12:15-12:30	**GENNARO DI PRISCO** Impact of pesticides on honeybee immunity and health
12:30-12:45	**LARA BOSCO** Side effects on *Orius* spp. of insecticides used on pepper and strawberry crops in northwestern Italy
12:45-13:00	**FILOMENA GRASSO** Olive tree genetic diversity and olive oil flavours
13:00-13:15	**SARA BORTOLINI** Insect colonization: carcass with fur vs carcass without fur
13:15-14:30	**LUNCH**
14:30-22:30	**Visit to the city of Torino**

Friday 9th November

Session 5: Insect Ecology and population dynamics	
8:45-9:30 **Plenary talk**	**KONRAD FIEDLER** Dissecting insect megadiversity: evidence from a tropical montane hotspot region
9:30-10:00 **Plenary talk**	**MARIA CRISTINA LORENZI** Chemical ecology of nestmate recognition in social wasps
10:00-10:05 **Poster**	**MARIA ELENA BONELLI** Geographic differences in the chemical profiles of a social wasp
10:05-10:10 **Poster**	**SANTOSH REVADI** Insights into the complex biology of an invasive *Drosophila* pest, *Drosophila suzukii*
10:10-10:25	**FRANCESCA BARBERO** A social parasite exploits cues from the coevolutionary race between food plant and host ant
10:25-10:55	**Coffee break**
10:55-11:10	**ALESSIO VOVLAS** Oviposition-site selection in *Zerynthia polyxena* (Lepidoptera, Papilionidae) and its implication for conservation
11:10-11:25	**NANA GRATIASHVILI** Taxonomy, behavior and life history of the endemic, slave-making ant *Myrmoxenus tamarae*
11:25-11:40	**DIEGO J. INCLAN** Impact of habitat fragmentation on the diversity of Tachinidae (Diptera) inhabiting semi-natural grasslands
11:40-11:55	**JORIAN PRUDHOMME** Spatiotemporal dynamics and bio-ecology of sandflies in the region of Montpellier
11:55-12:10	**DAVIDE RASSATI** Exploring introduction pathways of alien xylophagous insects in Italy
12:10-12:25	**FEDERICO LESSIO** Sampling and spatial distribution of phytoplasma vectors in the vineyard agro-ecosystem
Closing	
Lunch and departure of the participants	

Keynote speaker

Focus on arthropod kinships: hexapods and crustaceans thought to be cousins but eventually realized they are brothers

ANTONIO CARAPELLI

Department of Evolutionary Biology, University of Siena, Siena, Italy

Although age alone is not sufficient to justify evolutionary success, arthropods are undoubtedly the most booming animal lineage, displaying unmatched measures of evolutionary diversification, frequently measured as species number (i.e., 1 million of described living insects *vs* 5 million estimated), biomass, ecological impact, and so on. (At least) 525 MY of existence is a large amount of time during which the processes of morphological and genetic diversification among arthropod lineages have occurred. The traditional view on inter-relationships was well established essentially according to morphologically-based studies performed until the end of the last century. Thus, Chelicerata (scorpions, mites, spiders and allies) were treated as a separate group with respect to Mandibulata (a subclass, including Hexapoda, Myriapoda and Crustacea). Within this latter group, Myriapoda was, in turn, considered sister-group to Hexapoda or a grade from which hexapods evolved. Despite this latter controversy, insects and myriapods were universally grouped within the monophyletic taxon Atelocerata due to a large number of (presumed) synaphomorphies. Since the late 1990s onwards, by the time molecular techniques became popular science in systematic studies, phylogenetic relationships among arthropod main groups have been reinterpreted, frequently increasing the conflict between morphological- and DNA-based data. Lately, some morphologically anomalous results, such as the newborn relationships between Crustacea and Hexapoda (Pancrustacea), have become established time after time. Within Hexapoda, phylogenomic studies (mostly focused on mitochondrial DNA data) have been performed on basal key-groups, including those that never organized a complete set of flying devices (Apterygota). These results have boosted additional discussion centered on the presumed reciprocal paraphyly of hexapods and crustaceans, arousing opposite opinions on the issue according to the applied molecular marker.

Keynote speaker

Basic science in an applied world: insights on the leafhopper transmission of a plant pathogen

RODRIGO P. P. ALMEIDA

Department of Environmental Science, Policy and Management, University of California, Berkeley, CA94720, USA

Recent technological advances have allowed scientists to pursue novel questions in biology, as well as integrate knowledge originating from different disciplines. We will discuss new insights in the vector transmission of the bacterium *Xylella fastidiosa*, a bacterium of grapevines and many other crops of agricultural importance, as an example to illustrate how basic knowledge can be applied to various applied contexts, including potential responses to climate change, novel approaches to control diseases and others. We have recently addressed questions on how vector behavior affects disease spread, and will discuss these results in the context of climate change and other scenarios relevant to the management of vector-borne diseases. In addition, research on the vector-pathogen molecular interactions have yielded enough information on protein importance for *X. fastidiosa* colonization of leafhoppers, permitting the testing of a novel idea to block transmission, which has been demonstrated to be feasible through proof-of-concept experiments. This concept represents a new alternative to the control of vector-borne diseases. The integration of basic ecological, behavioral and molecular features of this system is allowing for the design of novel disease control strategies.

Plenary talk

Juvenile hormone titer and allatoregulating neuropeptides in wood-feeding cockroaches and primitive termites

KLAUS H. HOFFMANN, FRANZISKA WENDE

Animal Ecology I, University of Bayreuth, 95440 Bayreuth, Germany

Termites are eusocial insects with differentiated castes. They have two developmental systems, the linear pathway system or the bifurcated system, and JH plays a central role in caste regulation. Several studies have shown that JH titers are elevated in the course of soldier development. The synthesis of JH in the corpora allata of insects is regulated by allatostatins (AST) and allatotropins. Allatostatins of the FGLamide type (A-type) occur in many insect orders, but are known to inhibit JH biosynthesis only in cockroaches, crickets, and termites. The gene for the AST-A preprohormone has been identified in several cockroaches, but only in one termite species, *Reticulitermes flavipes*.

We identified the gene for the prepro-AST peptides in the only genus of the basal termite family Mastotermitidae, *Mastotermes*, species *M. darwiniensis*. In addition, the expression of the gene in brain and gut of all developmental stages and castes is demonstrated. The gene codes for 14 individual peptides and its sequence is most similar to that of the xylophagous wood roach, *Cryptocercus darwini*. There is a strong negative correlation between expression of the AST-A gene in the brain and the haemolymph JH III-titer in different developmental stages of the fertile and the sterile line of *M. darwiniensis* (measured by HPLC-MS). Knock-down of the AST-A gene by RNA interference, however, did not affect the JH titer of female and male workers. Our results confirm wood-feeding cockroaches as direct ancestors of termites, as well as the FGLamides as true allatostatins in both insect groups.

Plenary talk

Midgut remodeling in lepidopteran larvae: the contribution of autophagy and apoptosis

GIANLUCA TETTAMANTI[1], ELEONORA FRANZETTI[1], YANG CAO[2], QILI FENG[3], SILVIA CAPPELLOZZA[4], MORENA CASARTELLI[5], MAGDA DE EGUILEOR[1]

[1]Department of Biotechnology and Life Sciences, Università dell’Insubria, 21100 Varese, Italy; [2]Guangdong Provincial Key Laboratory of Agro-animal Genomics and Molecular Breeding, College of Animal Science, South China Agricultural University, Guangzhou 510642, China; [3]Guangdong Provincial Key Lab of Biotechnology for Plant Development, School of Life Sciences, South China Normal University, Guangzhou 510631, China; [4]CRA-Honey Bee and Silkworm Research Unit, 35143 Padova, Italy; [5]Department of Biology, Università degli Studi di Milano, 20133 Milano, Italy

Cell death phenomena occur extensively during the development and metamorphosis of holometabolous insects to eliminate tissues and organs typical of the embryonic or larval life. In Lepidoptera, the current literature is basically fragmentary and confusing and a clear understanding of the death mechanisms that intervene in several larval organs during metamorphosis is not available yet. With the aim to overcome this fragmentation of knowledge, we analyzed the remodeling process that takes place in the larval midgut of the silkworm, *Bombyx mori*, during metamorphosis and performed a detailed analysis of the cell death mechanisms in this organ.

To characterize the cell death processes that lead to the demise of the larval midgut epithelium, we investigated morphological, cellular and biochemical features typical of autophagy and apoptosis, and performed Real-time quantification of genes involved in the regulation of these two processes. Both autophagic and apoptotic markers showed appreciable changes in midgut cells at different stage of degradation.

Our data demonstrate that the degeneration of the larval midgut cells begins at wandering-spinning stage and it is a gradual process due to the concerted action of autophagy and apoptosis. Autophagy starts when the larva ceases to feed and it appears to be a pro-survival process able to recycle molecules from the degenerating cells and supply nutrients to the animal during the non-feeding period. Apoptosis intervenes later and it is actually responsible for cell death and for the disappearance of larval midgut cells.

Hsp70: studies on expression/overexpression in vivo in *Drosophila melanogaster* during rapid cold hardening (RCH) and after exposure at low temperatures

M. CAMEROTA, P.F. ROVERSI

CRA – Centro di Ricerca per l’Agrobiologia e la Pedologia – Firenze, Italy

Heat shock proteins (Hsp) are constitutive and inducible genes belong to molecular chaperones. They are involved in protein folding process. Many inducible Hsp are up-regulated in response to environmental stresses such as heat and heavy metals. Moreover, their involvement in cold stress is recently established. Hsp70 interacts with networks of other Hsp but it plays a key role during cold response. There are freeze-susceptible and freeze-tolerant insects and same factors such as anti-freezer proteins, lipoproteins, low-weight molecules as glycerol and threalose, increase supercooling capacity of freeze tolerant insects. *Drosophila melanogaster* is freeze-susceptible, no larvae survived when exposed directly to subzero temperature but an acclimation of larvae at low temperatures, called rapid cold hardening (RCH), caused an increased percentage survival before subzero exposure. Hsp70 expression increased at 0°C after RCH. In this work the focus will be on *D. melanogaster*: the time course expression of Hsp70 during larvae‘s RCH and at low temperatures will be evaluated. We will test whether the overexpression of Hsp70 influences the supercooling point and increases the percentage of adult survival. We will investigate also the effects of treatments on embryo ultrastructure.

Consequences of environmental stresses on male fertility, physiological and molecular aspects in the model *Nasonia vitripennis*

MARLENE CHIRAULT

Institut de Recherche sur la Biologie de l'Insecte, Université François-Rabelais, CNRS UMR 7261, Parc Grandmont, Tours, France

In recent decades, several studies have shown a decline in sperm production and quality in humans and wildlife. It was shown in our laboratory that the reproduction of hymenopteran parasitoids was also sensitive to environmental disturbances (Lacoume et al. 2006, 2007, 2009). Indeed, after various environmental stresses (food, chemical, thermal) on larval or pupal stages, the same phenotype is observed: a reduction in the amount of sperm in the seminal vesicles of males at emergence. Although these males as adults continue to produce sperm, the sperm stock remains deficient. This suggests that it is not the dynamics of production that is affected but the initiation of spermatogenesis. Such males are described as subfertiles. To understand mechanisms a model whose genome was sequenced recently will be used in my PhD: *Nasonia vitripennis*. Thermal and chemical stresses will be applied during the pupal development to measure the effects on male fertility by physiological, molecular, endocrine and metabolic analyses. We will try to identify whether the same mechanism is responsible for subfertility induced by different stresses. Mediators involved in the control of spermatogenesis will be screened on control and stressed males by molecular, metabolic and endocrine analyses.

Physiological and behavioural assays to explore host plant selection in two insect systems

BARBARA L. INGEGNO[1], GIULIA C. MAUTINO[1], JUAN ANTONIO SANCHEZ[2], LUCIANA TAVELLA[1]

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Plants produce a wide range of volatiles, varying quantitatively and qualitatively, that may act as signals, i.e. allelochemicals mediating interactions between insects and plants. Therefore, the volatile organic compounds (VOCs) released by plants influence the behaviour of numerous insect species, both phytophagous and zoophagous. To analyze the process involved in plant selection it should be investigated how the complex of environmental stimuli is processed by insects. The olfactory process can be divided into two phases: perception and selection. The perception is based on the physiological sensitivity, measured with electroantennographic (EAG) assays, while the selection is subjected to the subsequent interpretation of the message that triggers a mechanism of choice, and assessed with olfactometric assays.

This approach can be used to analyze host plant selection in insects with different feeding behaviour, e.g. the two generalist predators *Macrolophus pygmaeus* and *M. melanotoma* (Hemiptera, Miridae) and the phytophagous *Frankliniella occidentalis* (Thysanoptera, Thripidae). Both *Macrolophus* species responded to VOCs emitted by plants; however, even if almost all the tested plants were perceived, only their specific natural hosts were chosen in olfactometer. This demonstrated that peripheral level and central nervous system are both involved in the host plant selection process. EAG and olfactometer exploitation to test the system *F. occidentalis*–host plants is in progress. Based on the preliminary results, *F. occidentalis*, well-known for its polyphagy, responded to VOCs emitted by different solanaceous plants; nevertheless, its preference was closely related to the phenological stages of plants.

Genetic characterization of the urticating system in processionary moths of the genus *Thaumetopoea* (Thaumetopoeinae, Notodontidae)

LAURA BERARDI, ANDREA BATTISTI, ENRICO NEGRISOLO

DAFNAE-Entomologia, Università di Padova, Agripolis, 35020 Legnaro (PD), Italy

Larvae of the *Thaumetopoea* genus produce urticating hairs (setae) in the third and later instars on the dorsal part of the abdomen to protect themselves against predators. Contact with setae induces skin allergic reactions in humans.

The presence of a protein with allergenic activity (Thap2) has been demonstrated in the pine processionary moth *Thaumetopoea pityocampa* and opens the possibility of a comparative study aiming to identify and characterize the genes involved in the defense mechanism in all the *Thaumetopoea* species. The steps of the study are: characterization of Thap2 gene in species of *Thaumetopoea* genus, study of the Thap2 gene expression profiles, bioinformatic analyses and creation of full-length cDNA libraries using the RNA extracted from larvae.

We were able to amplify and sequence the Thap2 gene in several *Thaumetopoea* species as well as in *Ochrogaster lunifer*, an Australian thaumetopoein moth that has urticating hairs solely in the adult stage. This latter species, was used as an outgroup in the phylogenetic analyses. The Thap2 gene was present in all tested species.

The comparison of the protein sequences was done with various bioinformatic programs. Single nucleotide polymorphisms (SNPs) were observed in the sequence of the majority of species. These changes were mirrored by the presence of different amino-acids in the Thap2 protein of four *Thaumetopoea* species. Further bioinformatic studies will be devoted to understand the evolution of these genes in processionary moths.

The study may contribute to the development of antigens for testing the allergenic power of the urticating setae in humans.

Regulation of digestive enzymes in the Mediterranean field cricket *Gryllus bimaculatus*

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The Mediterranean field cricket, *Gryllus bimaculatus*, is an omnivorous insect which lives in meadows or dry fields. The main food consists of grassroots, fungi and insects (dead or alive). The food consumption of crickets is correlated to the developmental stage, age, sex and light-dark cycle. *G. bimaculatus* increases locomotory and feeding activity with the onset of darkness. Because crickets do not show a complete metamorphosis, they are able to regulate their digestive enzyme release continuously during both larval and adult development in response to changing environmental conditions. Apart from the essential protein digestion, *G. bimaculatus* has to deal with high contents of dietary cellulose and/or chitin and may encounter plant material containing enzyme inhibitors.

Many environmental and endogenous factors have been implicated in the control of digestive enzyme release in various insect species, but a comprehensive examination of all factors in a single insect species is lacking. Such an overview on the regulation of enzyme secretion will undoubtedly provide valuable information regarding adaptation of an insect to its environment.

Drosophila melanogaster as model system to study an evolutionarily conserved gene involved in insect development and immunity

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The CG2145 gene is a *Drosophila* homologue of the *Heliothis virescens* 102 gene, which is involved in the moth encapsulation response against invading pathogens and parasites. The spatio-temporal expression pattern of this gene during *Drosophila* embryogenesis was investigated by in situ hybridization experiments. In early embryonic stages, the CG2145 transcript was detected in cells undergoing major morphogenetic movements during gastrulation: ventral and cephalic furrow formation, amnioproctodeal invagination, anterior and posterior midgut development. Hybridization signals persisted in both mesodermal and endodermal layers throughout germ band elongation. Later on, the CG2145 gene was mainly expressed in immune tissues, ventral nerve cord and salivary glands. Interestingly, fat body and blood cells actively transcribed the CG2145 gene also in larval stages.

To study the function of the CG2145 gene by genetic means, we generated a set of random deletions spanning the gene locus. These deletions were obtained by imprecise excision of an EP element inserted in the 5’ UTR of the CG2145 gene, in a fly strain obtained from the Bloomington Stock Center. Deletion breakpoints were mapped by molecular analyses, leading to the identification of 11 different mutants. For 7 of them, the molecular lesion turned to be confined to the CG2145 gene. Western blot analyses using a polyclonal antibody directed against a recombinant protein produced in bacteria demonstrated that one of these mutant alleles was a null allele. Phenotypic analyses of this mutant are currently in progress.

Session 2: Insect behaviour

Plenary talk

Vibrational communication in insects: entangled and invisible networks on plants

VALERIO MAZZONI

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The communication by means of substrate-borne vibrations is the most spread channel of communication in arthropods, although at the same time the least studied and understood. In mating communication, vibrational signals allow the expression of many behavioural traits that also can constitute solid elements of individual fitness. A species can produce a more or less wide repertoire of signals, which are characterized by certain features (spectral and temporal) that eventually drive the mate choice and, consequently, the reproductive success. Signals are also decisive in intra-sexual competition (e.g. male-male rivalry), in inter-species or antagonistic interactions (e.g. predators or parasitoids that eavesdrop mating signals) and are affected by substrate characteristics (e.g. host-plant features). All these vibrational signals create a complicated network that travels along the substrate even for several meters. Any individual has its own Active Space Network (ASN), thanks to which it can interact with the surrounding world. The ASN can be defined as the cladogram-like substrate network covered by an active signal that spreads from a vibrational source. To describe and understand the many ASN occurring on the plants is a hard task and a researcher must respond to many questions. In first instance, he/she has to “learn” a signal, to distinguish between species-specific and individual-specific features, to insert it into the appropriate context, both behavioural and environmental. Only from the knowledge of the behaviour and the related communication system of a species it will be possible to set a reliable strategy of crop protection.

Vibrational signals as a prezygotic reproductive barrier

MAJA DERLINK, PETRA PAVLOVČIČ, MAARTEN DE GROOT, META VIRANT DOBERLET

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Substrate–borne vibrations are an important communication channel in many insect groups. We are interested in the role of these signals in the process of mate recognition and consequently their role as reproductive barriers. Our study organisms are leafhoppers of the genus *Aphrodes* (Hemiptera, Cicadellidae), in which currently established taxonomy has been unreliable. Testing female preferences has confirmed four and found a putative fifth species. Supporting mitochondrial COI marker and interspecific crossings suggest an important role of vibrational signals in maintaining reproductive isolation.

Acoustic behaviour in myrmecophilous insects

MARCO SALA[1], FRANCESCA BARBERO[1], LUCA PIETRO CASACCI[1], ANDREA DI GIULIO[2], EMANUELA MAURIZI[2], EMILIO BALLETTO[1], SIMONA BONELLI[1]

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About 10,000 arthropod species live as social parasites of ants and have evolved a number of mechanisms allowing them to penetrate and survive inside ant nests. Many of these parasites can intercept and manipulate their host’s communication systems. Recently we demonstrated that, in the *Maculinea*/*Myrmica* system, acoustic as well as chemical signals are used to penetrate and live inside the host ant colony. Within the ant nest, the *Maculinea* larvae lead a parasitic lifestyle and develop according to two different food strategies: *M. teleius* larvae will actively feed on the ants’ brood (predatory species) while *M. alcon* larvae will be directly fed by the worker ants by trophallaxis (cuckoo feeders). Here we compare the acoustical patterns of two *Maculinea* species: "predator” and “cuckoo” by using a high tech recording device which allows us to record unstressed insects.

We used the same method to investigate the capability of *Paussus favieri* (Coleoptera, Carabidae) to emit sounds and the role of these stridulations both during courtship and in modulating interactions with various castes of the host-ant, *Pheidole pallidula*. We demonstrated the parasite ability to mimic its host’s acoustic code and thus to interfere with its communication system. We finally point out the generally underestimated complexity and the importance of the ants’ acoustical channel, possibly modulating chemical cues and/or caste-specific behavioural responses.

How stress affects the mating behaviour in the leafhopper species *Aphrodes makarovi*

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Since reproduction is fundamental for species existence, it represents a part of necessary energy consumption for all organisms. For leafhoppers of the genus *Aphrodes* it is known that mating sequence starts with emission of male vibrational signals. When the male–female vibrational duet is established, male begins to search and localizes the female. To determine how the stress affects reproduction in *A. makarovi*, males were exposed to the two stress factors. A male–female duet was played to a group of non-starved males to test the effect of rival presence. The other group was starved and later subjected to the same duet. Single fed males duetting with a female were taken as a control. We compared the proportion of searching males, number and duration of male calls during the search, time spent to locate the female and success in locating the female within all three groups. In another set of experiments we measured oxygen consumption. Results showed that singing is energetically demanding. There were some clear differences in some aspects of sexual behaviour between males exposed to rivalry and those that were not and between starved and non-starved ones. Interestingly the male call duration was not changing within groups, indicating that it might be under the influence of sexual selection.

Olfactory response of a specialist and a generalist phytoseiid mite to cues emitted by tomato under combined infestation

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To initiate indirect defense, a plant under herbivore attack synthesizes and releases complex blend of volatiles that attract natural enemies of the herbivore. Infestations by multiple herbivores having different feeding guilds stimulate plant defense pathways in different manners compared to a single herbivore. However, to implement control strategies, more data are needed about the response of predators perceiving cues from plants under multiple infestations. Here, we report how the combined action of the phytophagous mites, the tomato russet mite *Aculops lycopersici* and the tetranychid mite *Tetranychus urticae*, affects the olfactory response of two different life style type predator mites: the specialist *Phytoseiulus persimilis* and the generalist *Neoseiulus californicus*. Trials were conducted on tomato plants, with a Y-tube olfactometer. The Herbivore Induced Plant Volatiles (HIPVs) blend from plants exposed to multiple and single herbivores were analysed by gas chromatography-mass spectrometry (GC-MS). The specialist *P. persimilis* showed a quick and stronger response to volatiles emitted under double infestation than to those of a single herbivore, whereas the generalist *N. californicus* did not show any immediate preference. The quantitative analysis of volatiles revealed that the amount of HIPVs was significantly higher in the case of a double herbivore attack. Expression analysis of the main genes of defense pathways is under investigation to understand how simultaneous infestations of a plant affect the olfactory response of two different phytoseiid species. Furthermore, the occurrence of other HIPVs possibly involved in this response is under consideration.

The kinematic of ants walking on the sand and in the antlion trap

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Among predation strategies, some sit-and-wait predators build a trap to capture preys. The trap can notably increase the attack’s radius and the probability of capture.

Some antlion larvae (Neuroptera, Myrmeleontidae) build an unstable conical trap in sand and wait at the bottom for a prey falling down. This trap retains preys within the range of the antlion because of both solid and liquid behaviours of sand. Indeed, when a prey tries to climb the solid slope, the sand can give way under the legs like a treadmill. However, different preys have different difficulties in view of this treadmill. The aim of this study was to understand how ants, the main antlion prey, can leave the trap. We firstly characterized the typical gait and the main kinematic properties (trajectory, velocity, step length) of ants walking on flat sand surface thanks to a high speed camera. Secondly we compared these results with ants failing to walk as well as ants succeeding in walking forward in the trap of the antlion *Euroleon nostras*.

Session 3: Insect interactions

Plenary talk

Zyginidia mRNA sequencing, where is the feminizing *Wolbachia* acting?

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Wolbachia (Rickettsiales) infects 66% of insect species and the bacterium induces specific host reproductive alterations. In *Zyginidia pullula*, *Wolbachia* causes the feminization of males. In this work overwintering females of *Z. pullula* were grown in the laboratory and the exclusively female lines (index of *Wolbachia* infection) and the normal 1:1 sex ratio offspring were grouped into four categories: normal females, normal males, infected females and feminized males. Total RNA was extracted from the different categories and the messengers were used for the synthesis of the cDNA. The "high throughput" sequencing was conducted in collaboration with the Laboratory of Prof. Sergey Nuzhdin at the Department of Biological Sciences - Molecular & Computational Biology at the University of Southern California in Los Angeles.

The preliminary analysis of genes differentially expressed in different categories shows that, in the process of feminization, genes responsible for sex determination and development seem to be involved. One hypothesis could be that the bacterium is able to interfere with hormonal biosynthetic pathways that involve insulin and ecdisteroids, being these hormones involved in the processes of development and sexual differentiation.

Genomic adaptation of the parasitoid *Cotesia sesamiae*

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The wasp *Cotesia sesamiae* is a parasitoid of corn borer caterpillars (e.g. *Sesamia nonagroides*, a pest species with African origin and invasive in France). In the prospect of biological control, various studies have revealed that natural African wasp populations show specialization based on local host species. This local adaptation is partly correlated with specific forms of a symbiotic virus (polydnavirus) integrated in the wasp genome during evolution. Indeed, wasps inject into the body of the caterpillar during oviposition, polydnavirus particles containing virulence genes in order to interfere with the immune system of their hosts. During this study, the changes in the entire genome of the wasp and in particular in the integrated provirus genome are being studied in detail. This will allow discovering which genes are involved in the adaptation to the lepidopteran host species but also in the adaptation to geographical and climatic conditions. The first step will be the acquisition of a reference genome. However, *C. sesamiae* is not the best choice for obtaining a genome assembly due to its high genetic diversity. By contrast, a highly inbred laboratory strain of the congeneric species *Cotesia congregata* is available and ideal for obtaining a reference genome. The second step will be the high-throughput sequencing (RAD-tag) of genomes from adapted populations of *C. sesamiae*. Various approaches will allow identifying candidate genes. Understanding the mechanisms of adaptation should give keys to select effective strains usable as biological control agents.

Dissecting the effects of fusion phytoplasma membrane proteins on vector acquisition and transmission capabilities

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Phytoplasmas are unculturable plant pathogens transmitted by hemipteran phloem-sucking insects. Studies on ‘*Candidatus* Phytoplasma asteris’ (CYP) showed that the antigenic membrane protein (Amp) interacts specifically with vector membrane proteins. Following acquisition, phytoplasmas must cross the gut epithelium and colonize salivary glands for successful transmission. The gut epithelium and salivary glands are physical barriers that must be overcome by the phytoplasma during vector colonization. Specific interactions of Amp with vector membrane proteins occur *in vitro* at both sites. Phytoplasmas cannot be grown axenically and this hampers further studies on the *in vivo* interactions between phytoplasma and vector membrane proteome. The aim of this work was to develop a system to study the effects of phytoplasma membrane proteins on vector acquisition and transmission capabilities. To investigate the biological effects of the interactions of CYP Amp at the gut epithelium, vectors of two species (*Macrosteles quadripunctulatus* and *Euscelidius variegatus*), before acquisition on CYP infected plants, were fed on a medium containing: a fusion construct of the protein; a fusion protein of ArtI, a membrane protein not involved in specific interaction with vectors; antibodies raised against Amp and ArtI; no proteins. After latency, insects were singly caged on healthy plants for an inoculation period after which they were collected and assayed in nested PCR to detect the presence of CYP. The phytoplasma titre in insects was also quantified by RT-PCR. Inoculated plants were observed for symptom appearance and their sanitary status was confirmed by PCR. The results of preliminary experiments are presented and discussed.

Phylogenetic relationships between European and Hawaiian species of the genus *Trupanea* (Diptera, Tephritidae) and their symbiotic bacteria

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The genus *Trupanea* belongs to the subfamily Tephritinae (Diptera, Tephritidae), the most specialized subfamily of fruit flies, the larvae of which feed on Asteraceae hosts predominantly in the flower heads. Tephritinae species are known to host specific non-culturable symbiotic bacteria (‘*Candidatus* Stammerula spp.’) in the midgut. In the Hawaii islands the presence of about 20 different species has been reported, belonging to the genus *Trupanea* and infesting Asteraceae.

In this work we studied the association, from an evolutionary and phylogeographic perspective, of the Hawaiian endemic *Trupanea* species (collected in different islands) and their endosymbionts comparing with European species. Two regions of the mitochondrial DNA (16S rDNA and CO) of the flies were examined while the 16S gene was analyzed to study the symbiotic bacteria.

Up to date 9 *Trupanea* species have been studied and more 8 are being studied now. Preliminary analyses reported that in all the phylogenetic trees (insect hosts and symbiont) the Hawaiian and the European species belonging to the genus *Trupanea* formed a strongly monophyletic group highly supported. The presence of phylogenetic congruence between flies and symbionts will be investigated.

Impact of leaf-miner insects on the primary metabolism of their host-plant: manipulating from the inside

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Endophytophagous insects, such as stem-boring, gall-forming and leaf-mining insects, live within plant tissues and feed internally. This life-style presumably provides adaptive advantages for the insect over other external-feeding modes by allowing access to most nutritional tissues while avoiding main plant defensive compounds. This selective-feeding behaviour can be reinforced by manipulating the plant physiology, as suggested by the formation of ‘green-islands’ around mining caterpillars in yellow leaves.

We study this so-called ‘nutrition hypothesis’ on the system *Malus domestica*/*Phyllonorycter blancardella*. Thanks to colorimetric assays, capillary electrophoresis and GC/MS, we quantified proteins, starch, total soluble carbohydrates, main individual carbohydrates, free and protein-bound amino acids on green and yellow mined leaves.

Our results suggest an active manipulation of the plant primary metabolism by the leaf-miners with a plant response to insect herbivory strongly dependent on the feeding mode of the larva. Indeed, this species is characterized by a larval hyper-metamorphosis with first instars being fluid-feeders and last instars being tissue-feeders. Tissue-feeding larvae control their micro-environment in order to generate a microenvironment with all the nutrient supply needed for their growth and survival but fluid-feeding larvae seem to be limited in such capacity to manipulate the plant physiology. All together the observed plant metabolic changes in the mine further strengthen the hypotheses that mines behave independently from the hosting leaf, operating a metabolic machinery on their own. By isolating the mine from the rest of the leaf, the insect buffers environmental variations of plant quality, presumably protects itself from plant defenses and obtains a nutritional autonomy.

Molecular basis of plant gall induction by cynipid wasps

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Cynipid wasps lay their eggs in host plants and induce the formation of spectacular galls with complex morphologies that are specific to the inducing wasp species. Induction of these new plant structures –absent in healthy plants – implies a tight manipulation of the host plant by the insect parasite. These galls constitute perfect examples of extended phenotypes: the development of these galls composed of plant tissues is completely controlled by the parasitic insect.

The ecology of gall wasps and the diversity and morphology of the impressive structures they generate on plants have been well documented and have spurred different hypotheses on the adaptive significance of gall induction. Surprisingly however, the molecular basis and therefore the underlying mechanisms of gall induction remain unknown.

To identify candidate gall-induction effectors, we have initiated a high-throughput transcriptomic approach on the venom gland and ovaries of two cynipid gall wasps, *Biorhiza pallida* and *Diplolepis rosae*, that induce galls on oak and dog-rose, respectively. These two organs were chosen because in parasitoid wasps they are known to produce virulence factors that modify the insect host physiology. Pyrosequencing of venom gland and ovary cDNA libraries in these two galling species has generated 597,290 reads of 350 bp average length. Here, we shall present the initial analysis of the sequence data. This approach constitutes the first molecular investigation of galling insect-plant interactions and should allow the identification of candidate insect effectors involved in host plant manipulation.

First functional annotation of a polydnavirus: the expressed *Cotesia congregate* bracovirus explored

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Cotesia congregata (Hymenoptera, Braconidae) develops as a gregarious endoparasitoid inside larvae of the tobacco hornworm *Manduca sexta* (Lepidoptera, Sphingidae). The parasitoid wasp has evolved virulence strategies using a viral obligatory symbiont of the Polydnavirus (PDV) family named *Cotesia congregata* bracovirus (CcBV). CcBV particles are produced by specialized cells of the wasp ovaries and are injected along with the eggs into the host body.

The PDV genome exists as two distinct forms: (i) a linear symbiotic form that is integrated into the wasp’s genome and vertically transmitted to its offspring; (ii) an encapsulated circular pathogenic form that is injected into the host by the wasp during oviposition. The integrated genome of the encapsulated virus is made up of 36 segments grouped into 9 regions of the wasp genome and encodes 252 predicted genes distributed in 34 multigenic families.

In the wasp, the viral gene expression mainly concerns genes involved in viral particle production. In the caterpillar host, the expression of only a few selected candidate virulence genes had been studied, and so far we lacked a global vision of viral gene expression, in particular concerning genes belonging to gene families.

Here we performed a large-scale transcriptomic analysis of two distinct immune tissues (fat body and hemocytes) of the host *M. sexta* parasitized by *C. congregata*. Following this analysis, we were able to identify 76 CcBV genes expressed 24hrs after parasitism. This analysis allows us for the first time to have a snapshot of global viral gene expression during parasitism at one given time and in two tissues. In particular, we could show differential gene expression of genes belonging to a same gene family. This type of analysis will help us to highlight viral virulence genes that play an essential role in the host-parasitoid interaction.

Ultra-deep sequencing of AcMNPV and comparison to original genome sequencing

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Natural selection relies on genetic variation to lead to ecological adaptation. Baculovirus occlusion bodies containing numerous genomes foster the maintenance of genetic diversity. This diversity, characterised by restriction endonuclease profile or gene sequencing, has long been known in AcMNPV. Here, we present a novel approach to assess the diversity harboured in the original P.Vail isolate using Illumina© ultra-deep sequencing techniques. We obtained a 176,000X genome coverage, allowing solid statistical tests on the genetic variation. Our isolate shows a 0.2% pairwise difference with the clone C6, from which it derived. However, in our isolate we found 1 million mutations over the 133,926 bp long genome. SNPs were found ready to switch with the consensus in some loci, and in others where several variations are found, in genes involved in adaptation.

This approach provides precise information on the diversity in this isolate, with modulation of genes involved in essential pathways.

Cytoskeleton proteins of the insect vector are involved in phytoplasma infection

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Phytoplasmas are transmitted by leafhoppers, planthoppers and psyllids in a persistent propagative manner. Pathogen membrane proteins are in direct contact with hosts and are presumably involved in determining vector specificity. The major phytoplasma antigenic membrane protein (Amp) specifically interacted *in vitro* with few vector proteins (actin, α and β subunits of ATP synthase), and is hypothesized to play a crucial role in the relationship with the vector. Biological effects of phytoplasma Amp on insect acquisition and transmission capabilities are under study. To unravel phytoplasma-vector interactions, we compared proteome profiles of healthy and infected adults of *Euscelidius variegatus*, vector of *Chrysanthemum yellows phytoplasma* (CYP, '*Candidatus* Phytoplasma asteris'). In three biological replicates for each treatment about 400 spots were identified, ten of which significantly diversely expressed. Peptide mass fingerprinting identified only one spot, more expressed in infected insects, matching with tropomyosin of *Drosophila melanogaster*. Mono- and bi-dimensional Western Blots with a commercial polyclonal antibody against insect tropomyosin confirmed that this protein was more expressed in infected *E. variegatus* and *Macrosteles quadripunctulatus*, another CYP-vector. *E. variegatus* and *M. quadripunctulatus* tropomyosin cDNA were cloned and sequenced; transcript profiles in qPCR and sequence features of the gene were determined too. Tropomyosin is an actin-binding protein, involved in cytoskeleton organization. Contraction of muscle tissues, vesicle trafficking, endocytosis and other functions have been ascribed to many different tropomyosin isoforms of multicellular organisms, from human to yeast. A phytoplasma effect on cell host cytoskeleton could be hypothesized due to Amp interaction with vector actin and tropomyosin increase in infected insect cells.

Interactions between the phytoplasma '*Candidatus* Phytoplasma mali' and the vector *Cacopsylla melanoneura* (Hemiptera, Psyllidae) in the transmission of Apple Proliferation in NW Italy

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Apple Proliferation (AP) is an epidemic disease caused by the phytoplasma '*Candidatus* Phytoplasma mali' (APP), which affects apple trees of central Europe. In Northwest Italy APP is mainly transmitted by the psyllid *Cacopsylla melanoneura*. Even if previous works demonstrated the role of overwintered adults in the transmission and retention of the infection during the overwintering period, some aspects of the transmission associated with newly-emerged individuals still remain unclear due to the short period spent on apple trees before the migration to conifers for the aestivation and overwintering period. Since recent studies identified some aestivation and overwintering sites in Northwest Italy, the variation of the phytoplasma titre in all insect life stages was investigated. Thus some aspects of the transmission phases were better defined. For this purpose a Real Time quantitative PCR protocol has been developed for the specific detection and quantification of the APP titre in the insect vector. Then acquisition, latency and multiplication study trials with overwintered adults, nymphs and newly-emerged specimens were set up. The results obtained confirm the acquisition ability of both nymphs and adults, a life-long retention, and let suppose a quite long latency period. These data shed light on the epidemiology of the disease with practical consequences on the vector control management.

Plenary talk

New insights on tomato defence responses

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In tomato, systemin (Sys) is an 18-aa oligopeptide and is a primary signal involved in the activation of defence genes in response to wounding and herbivory. Sys is released from a larger precursor (ProSys) following wounding with an unknown mechanism and locally promotes the biosynthesis of Jasmonic Acid (JA), the molecule responsible of the systemic wound signaling in tomato plants. Sys activity has been addressed to the induction of defences active against chewing insects. To shed more light on Sys role in tomato defence against phytopathogenic fungi and phloem-feeders, transgenic plants constitutively expressing ProSyswere produced (RSYS) and evaluated in their resistance/susceptibility by bioassays with *Botrytis cinerea* and *Macrosiphum euphorbiae*. RSYS plants resulted more tolerant than control plants to both stressors. The molecular basis of these observations were investigated through microarray analysis. Blast2GO software (CIPF, Valencia) was used for the functional annotation of differentially expressed sequences. Most of them are classified in "stress responses" and "signaling" categories: ProSys was found to affect the expression of genes related to defence against herbivores and pathogens, including genes involved in the reinforcement of physical barriers and responses to environmental stresses.

Moreover, the expression of genes related to the emission of volatile compounds, associated with indirect defences, such as phenylpropanoid and terpenoid, was also affected. Interestingly, genes involved in jasmonic acid, salicylic acid, ethylene and auxin-regulated pathways were differentially expressed, suggesting that Sys could influence the defence networks controlled by different plant hormones. Overall our results indicate that tomato Sys plays a key role in the coordination of plant defences against different stressors.

Session 4: Insect control and biotechnology

Plenary talk

Insect-bacteria symbiosis: a potential tool for pest control

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The symbiotic association between unicellular and multicellular organisms has provided a significant contribution to life evolution on Earth by improving the metabolic capabilities of eukaryotes. Symbiosis with bacteria is very common in invertebrates; moreover one of the classes which majorly took advantage of this interaction are the insects, which were able to occupy several ecological niches thanks to their interactions with microorganisms. Bacteria may play different roles in their insect hosts. Those that increase the host’s metabolism, by providing essential nutrients for insect survival, are called primary symbionts. These associations are obligate for both organisms and maternally transmitted; the symbionts are intracellularly placed into specific organs called bacteriomes. Beside primary symbionts, many insects host other bacteria, named secondary symbionts, not necessary for the insect’s life; nevertheless they ameliorate host fitness. Secondary symbionts, extracellularly located in different insect tissues, may be found with low frequencies in host populations even though they can establish permanent associations and be vertically transmitted. Another symbiont group is that of maternally transmitted sexual manipulators, which influence the host’s reproduction in order to increase the female progeny to promote their spread.

Considering the strong influence of bacterial symbionts on the hosts’ biology, the diversity of symbiotic species and strains may heavily affect insect development and reproductive capacity, with important ecological and evolutive implications. Such an impact could be exploited for the containment of pest insects and pathogens, by means of Symbiotic Control strategies. The potential of this approach will be described in detail.

Potential symbiotic control agents against *Drosophila suzukii*

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Drosophila suzukii (Diptera, Drosophilidae) is one of the most dangerous pests for fruit crops recently introduced into Europe from South-East Asia. The characteristic ovipositor of females allows to lay eggs into ripening fruits, which are damaged and become unsuitable for the market.

Due to the economic impact of this insect several studies concerning different defensive strategies to control the pest are ongoing. One of these strategies is represented by the symbiotic control. In fact, symbiotic microorganisms in insects play important roles on the vitality and also on the relationship between the host and other organisms of the same environment. Thus, the symbiotic control could represent an interesting strategy to contain the development and the spread of *D. suzukii*, however an intensive study is necessary to identify the main symbiotic microorganisms to be manipulated for the control of the pest. This study includes at first the identification and characterization of the whole microbial community of *D. suzukii*. After the identification of the microorganisms it is also important to understand their distribution in the insect body. If there is a high concentration of symbiotic organisms in salivary glands or in gonads, transmission tests will be necessary too. Finally, it is fundamental to evaluate the possible environmental risk of the symbiotic control agent including vegetable and animal hosts and other microbial communities present in the area.

The results of a preliminary characterization of the microbial community of *D. suzukii* will be presented.

Development of new tools for the integrated management of Flavescence dorée of grapevine and the vector *Scaphoideus titanus*

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Flavescence dorée (FD) is a serious epidemic disease of grapevine associated with phytoplasmas of '*Candidatus* Phytoplasma vitis', specifically transmitted from grape to grape by the leafhopper *Scaphoideus titanus* (Hemiptera, Cicadellidae). So far control measures have relied on insecticide foliar applications against the vector, the use of healthy propagation material and the rouguing of infected plants in the vineyard. In spite of all the efforts, the disease is out of control in some viticultural areas and negative side effects of insecticides have been reported, especially on bees. For these reasons, we aim to develop innovative protection strategies that include a new systemic insecticide delivery targeted to the grapevine wood and the application of synthetic and biotic resistance elicitors. Preliminary results on the application of thiamethoxam to the grape wood are presented. The insecticide activity was evaluated by bioassays against the vector and by using thiamethoxam-specific antibodies in a lateral flow test on grapevine leaves at different times after treatment. A correlation between insect mortality and insecticide concentration in the leaves was obtained. The next step will be the evaluation of synthetic (benzothiadiazole) and biotic (the epiphytic bacterium *Pseudomonas putida* and the mychorrizal fungus *Glomus mosseae*) resistance elicitors in protecting grapevines from FD transmission by the vector. The attractiveness to the vector of grapevine plants treated with synthetic and biotic elicitors will be studied by means of olfactometer and choice tests.

Gall wasp community assembly across historical and regional scales

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The evolutionary interaction of a particular group of insect herbivores, cynipid gall wasps (Hymenoptera, Cynipidae, Cynipini) and their parasitoids (Hymenoptera, Chalcidoidea) across a variety of historic and geographic scales has been investigated. The aim is to investigate the way in which communities assemble and interact by
1) assessing the interaction of native Mediterranean parasitoids with *Dryocosmus kuriphilus*, a recent invasive gall wasp from China, as well as with its introduced biological control agent, *Torymus sinensis*
2) testing the interaction of spatially divergent oak gall wasp communities on sky islands in south-western USA, and
3) examining the global phylogeography of inquilines (Cynipidae, Synergini) - a trophic guild comprising commensal relatives of the cynipids - within the gall wasp community.

Together these projects fill important gaps in our knowledge of cynipid gall communities. This presentation concentrates on the interaction of native parasitoids with the recent invader in Europe, *D. kuriphilus*, and its biological control agent, *T. sinensis*.

The natural enemy *Torymus sinensis* for the control of the chestnut gall wasp

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Dryocosmus kuriphilus (Hymenoptera, Cynipidae) is one of the most serious pests attacking chestnut trees. It is native to China and was incidentally introduced into Northwest Italy and it is now spreading throughout Europe. This pest was successfully controlled in Japan and USA by introducing a parasitoid, *Torymus sinensis* (Hymenoptera, Torymidae), from China’s mainland. Following these successful experiences, the parasitoid was introduced into Italy from Japan. *T. sinensis* is the only Chinese parasitoid species of *D. kuriphilus* so far known to be host specific, and phenologically well synchronized with *D. kuriphilus*. *T. sinensis* females lay eggs into newly formed *D. kuriphilus* galls in early spring, and the parasitoid larva feeds externally on the mature host larva until pupation during late winter.

Following the successful establishment of *T. sinensis* at the first release sites, a rearing programme was set up to support the release of *T. sinensis* at additional infested sites in Italy, and *T. sinensis* is currently being introduced into most regions across Italy, and to some parts of France. Results collected from the Italian release sites indicate that the rate of parasitism of *D. kuriphilus* galls is increasing faster and the first effectiveness, the reduction of gall wasp infection rates, is evident at several sites.

Parasitoid-inspired selection of a target gene for RNAi

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The bracovirus associated with the parasitic wasp *Toxoneuron nigriceps* (TnBV) is involved in the host immunosuppression syndrome, as it negatively affects the expression in *Heliothis virescens* host larvae of an important immune gene. This gene regulates both the humoral and cellular response, and its homologue is present in related lepidopteran species. Its RNA interference (RNAi) knock out, by oral delivery of dsRNA, has been performed both in *H. virescens* and *Spodoptera littoralis* larvae, generating immunosuppressed phenotypes, more sensitive to pathogen infection. Moreover, this gene is also highly expressed during embryonic development and its silencing mostly prevents egg hatching and determines a significantly higher mortality rate in new-born larvae. Therefore, it appears to be a very good candidate as a molecular target of novel pest control strategies, based on the use of RNAi technologies.

Causes of success and failure in biological control: venom contents and evolution in the parasitoid wasps *Psyttalia lounsburyi*/*P. concolor*

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The success of endoparasitoid wasps depends on their capacity to control the host’s physiology and immunity. Parasitoid virulence strategies are highly diversified, but most of them relies on injected venom proteins at oviposition.

This project aims to characterize the venom components of two hymenopteran parasitoid species (*Psyttalia lounsburyi* and *P. concolor*), to analyze variations between populations and individuals within species, and to follow their evolution in response to the use of a substitute host under laboratory conditions. The host change might indeed result in selection pressures leading to the adaptation to the new host, and maladaptation to the target host.

The braconid wasp *P. lounsburyi* is used as a biological control auxiliary against the olive fruit fly, *Bactrocera oleae*. However, the production of parasitoids is performed on the substitute host *Ceratitiscapitata* due to the difficulty of rearing *B. oleae*. We also work on a related parasitoid species (*P. concolor*), used against the olive fruit fly. *P. concolor* is more generalist than *P. lounsburyi* and is supposed to develop on both *C. capitata* and *B. oleae* in the field.

Preliminary results include i) the characterization of venom components by transcriptomic and proteomic approaches to identify some putative protein functions, ii) the assessment of the population and inter-individual variation in venom protein content using 1D SDS-PAGE profiles. Preliminary results suggest that individual variation might be greater in wild populations obtained from *B. oleae* in the field than in laboratory strains maintained on *C. capitata*.

How much venom is necessary for the success of endoparasitoid wasps?

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Endoparasitoid wasps lay eggs inside the body of other insects, causing their death. To prevent egg survival and development, hosts have evolved a highly efficient immune reaction, leading to encapsulation of the invader, but parasitic wasps can circumvent this response by injecting ovarian fluid and venom contents in the host at oviposition. Over the past decades, studies have focused on the nature and physiological functions of parasitoid venom, and numerous venom proteins have been identified. However, an accurate demonstration of their role in parasitization success is still precluded by our lack of knowledge on the real amount of the protein naturally injected by the wasp. This may indeed result in false estimations of *in vitro* or *in vivo* effects, if the assays are performed with a non-physiological amount of the protein (Colinet et al., 2011).

We have taken advantage of the detailed characterization of venom proteins from *Leptopilina boulardi*, a larval endoparasitoid of *Drosophila melanogaster*, to address, at the individual level, the question of the quantity of venom fluid and proteins injected by the wasp. This was performed by using an antibody raised against the main immune suppressive factor in this species, a RhoGAP protein demonstrated to be necessary for parasitism success. Finally, to question whether the quantity of venom injected in the same host may differ between closely-related parasitoids, we developed and similarly used an antibody directed against a major venom protein of *L. heterotoma*. Results are discussed in the context of the diversity of parasitization strategies.

Colinet et al. (2011) J. Biol. Chem. 286(46), 40110–40121.

Evaluation of a cryopreservation protocol for *Galleria mellonella* eggs parasitized by *Trichogramma brassicae*

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There is an increasing need for methods of cryopreservation of arthropods useful in agriculture; in particular, for Hymenoptera, which are extremely important in applied entomology, for their use in biocontrol and their importance in biodiversity conservation.
Starting from a cryopreservation protocol developed for *Galleria mellonella* eggs (Roversi et al., 2007; Cosi et al., 2010), we tried to adapt this protocol for hymenopteran egg parasitoids. For this work we choose the well-studied biocontrol agent *Trichogramma brassicae* because of its rapid life cycle and its wide host range.
Here, we will present results of a *G. mellonella* cryopreservation protocol applied on *T. brassicae* parasitized eggs.

Roversi P.F., Cosi E., Irdani T. (2007) Chill sensitivity and cryopreservation of eggs of the greater wax moth *Galleria mellonella* (Lepidoptera: Pyralidae). Cryobiology 56 (1): 1-7.
Cosi E., Abidalla M.T., Roversi P.F. (2010) The effects of tween 80 on eggshell permeabilization in *Galleria mellonella* (L.) (Lepidoptera Pyralidae). Cryoletters 31: 4 291-300.

Life table of *Oencyrtus pityocampae* reared on *Nezara viridula* eggs

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In many regions of the Mediterranean basin the lepidopteran defoliator *Thaumetopoea pityocampa*, the pine processionary moth, is a harmful forest pest responsible for severe damage to tree canopies of both natural and artificial coniferous stands. Its main targets are *Pinus* spp. and *Cedrus* spp. Furthermore, urticating hairs of larvae may represent a major public health concern in urban settings. In a context of pest management strategies, biological control by parasitoids is playing an increasing role due to the possibility of economical mass rearing and rapid release in the field of these biocontrol agents. Among hymenopteran endoparasitoids, the encyrtid *Ooencyrtus pityocampae* has proved to be a valuable tool for targeting *T. pityocampa,* because of its wide-range distribution, ability to develop on different hosts, efficacy and capacity to adapt to host density fluctuations. However, a limit to its employment in natural settings is represented by a general lack of knowledge of both biological parameters and behavioural traits and by the difficulty of storing parasitoid immature stages in natural and alternative host eggs. In this first contribution, *O. pityocampae*’s developmental biology and its reproductive parameters have been evaluated by means of microscopy and life table analysis.

Impact of pesticides on honeybee immunity and health

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Large-scale losses of honeybee colonies seem to have a multifactorial origin, with both biotic and abiotic stress factors being apparently involved. Among the latter, pesticides have been invoked as important agents inducing colony decline, with neonicotinoids often considered as key-players in this complex syndrome. However, the experimental data gathered so far are still fragmentary and further research efforts are therefore needed. Here we investigate the impact of neonicotinoids on the immune system of the honeybee in order to understand if and how these pesticides can promote the progression of silent viral infection, with negative consequences on colony health and stability. We observed that exposure to sub-lethal doses of neonicotinoids triggers intense replication of Deformed wing virus (DWV), while organophosphates did not. This effect was analyzed in the framework of the model we have recently proposed, based on the central role played by NF-kB transcription factors in the anti-viral immunity and in the activation of physiological responses to different environmental stress factors, using also drosophila as a model to assess the impact of neonicotinoids on NF-kB signaling in insects.

Side effects on *Orius* spp. of insecticides used on pepper and strawberry crops in northwestern Italy

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The species of the genus *Orius* (Hemiptera, Anthocoridae) are well-known as generalist predators able to control thrips outbreaks on different crops, such as pepper and strawberry. The study of the compatibility of insecticides with biocontrol agents is a key for the success of IPM strategies, including *Orius* releases on the crops. During 2011, experiments were carried out in Piedmont (northwestern Italy) in four commercial pepper and strawberry tunnels where *O. laevigatus* and *O. majusculus* were released. Despite the releases, the wild *O. niger* was the most abundant species on both crops, and naturally colonized tunnels starting from mid-June. Generally, the highest negative fluctuations of predator populations occurred after insecticide applications, in particular abamectin, etofenprox and λ-cyhalothrin. On the other hand, pirimicarb, spinosad and azadirachtin were apparently safer for anthocorid populations. Toxicity of the most used insecticides was also evaluated in laboratory bioassays on *Orius* spp. collected on wild flora in the surroundings of greenhouses, where *O. niger* was the predominant species. Abamectin, λ-cyhalothrin and acrinathrin proved to be incompatible with these predators, also in laboratory experiments. Azadirachtin was almost harmless for *Orius* spp. whereas spinosad induced high levels of mortality in laboratory bioassays. Under laboratory conditions etofenprox was less toxic than what expected from field experience, corroborating the need of multiple testing methods to evaluate the effects of pesticides on beneficial insects.

Olive tree genetic diversity and olive oil flavours

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Campania is one of the Italian regions most suited to the production of olive oil. Following previous studies and in order to provide relevant information for the conservation and enhancement of its olive germplasm and corresponding typical olive oils, we have been extending the descriptive framework of genetic variability at the 20 main varieties cultivated in Campania by means of Simple Sequence Repeats (SSR). Furthermore, exploring the correlation between SSR profiles and three olive oil quality traits (fatty acids, biophenols, sensory profile), a significant correlation between SSR profile and sensory profile has been shown.

An important aspect of olive oil quality is the plant's interaction with its key enemy, the dipteran *Bactrocera oleae*. Following its attack, host plants activate a cascade of genes, thus modifying the mixture of volatile compounds (VOCs) emitted and activating the defence mechanisms. In this framework, lipoxygenases (LOX) are involved in the production of VOCs from linolenic acid. Some of these play an important role in the typicity of extra virgin olive oils, so that is possible to hypothesize that the drupe production of some aromas is somehow modulated by the attack or the presence of the olive fly. VOCs emitted following the presence of *B. oleae* have been studied by means of GC/MS. Then I will perform transcriptomic studies to clarify the molecular mechanisms underlying the VOC production and finally investigate the correlation between the presence of the olive fly and the typical flavour of some monovarietal extra virgin olive oils of Campania.

Insect colonization: carcass with fur *vs* carcass without fur

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The entomologic approach is increasingly applied in legal cases regarding animals and veterinary problems. Several studies about insect colonization involving pigs (*Sus scrofa*) are available. This species is frequently used as a model for humans, but the most evident difference between this model and other terrestrial mammals is the lack of a dense fur. Little information about the effects of the fur on arthropod colonization is available. For this reason, the aim of this preliminary work was to investigate the effects of fur, season and fur x season on the colonization of animal carrions by insects. Rabbits (*Oryctolagus cuniculus*) were used as a model for animals with fur. Five experiments were performed during 2011 in a field in the neighborhood of Modena (northern Italy) (one in winter, one in spring, one in summer and two in autumn). For each test, two rabbits were used, one with fur and one without fur. The carcass without fur had been considered as control. Three Dipteran species were collected: *Calliphora vicina*, *Lucilia sericata* and *Lucilia illustris*. Hymenopteran parasitoids (*Tachinaephagus zealandicus*) and larder beetles (*Dermestes undulatus*) were also collected. No differences in the composition of the Dipteran fauna present on the two types of carcasses were detected. Conversely, a delay in the colonization was detected in the carcass with the fur in all the trials, especially in colder seasons. Furthermore, the fauna’s composition changed in the colder and warmer seasons, confirming the phenology of the genera *Lucilia* and *Calliphora* in northern Italy.

Session 5: Ecology & population biology

Plenary talk

Dissecting insect megadiversity: evidence from a tropical montane hotspot region

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Tropical mountain forests are hotspots of global biodiversity. For certain taxa, they may even surpass the better known lowland tropical forests with regard to species density. At the same time, these forests are under serious threat through increasing human land-use pressure and ongoing climate change. Using data from sampling adult moth communities by light-trapping, and from inventories of caterpillar assemblages on selected plants in the Andean rainforest zone in southern Ecuador, I will explore how diverse these ensembles indeed are and how this diversity varies across ecological gradients at small spatial scales (viz. elevation and succession). Different moth taxa, such as Pyraloidea, Geometridae, and Erebidae-Arctiinae, show a remarkable mixture of concordant as well as contrasting diversity patterns. Then, I will address the role that host plant specialism may play in shaping moth diversity, and how different functional types of caterpillars with regard to their feeding mode contribute to this diversity. Finally I will address the phylogenetic dimensions of moth diversity in tropical Andean forest, using on the one hand a phylogenetic reconstruction of one massive radiation (the geometrid genus *Eois*), and on the other hand the phylogenetic diversity of entire moth ensembles along an extended elevational gradient. These data will be used to assess whether Andean moth megadiversity has been predominantly shaped by geologically recent events (Pleistocene climate cycles and concomitant forest dynamics), or has accumulated over longer periods of evolutionary time (during the Neogene).

Session 5. Ecology & population biology

Plenary talk

Chemical ecology of nestmate recognition in social wasps

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Insect communication is a central topic in insect sciences. Much information is known about how insects respond to simple chemical signals but the question remains how insects use the complex information in multicomponent chemical signals. For example, in social insects, recognition signals often have > 50 epicuticular hydrocarbons which communicate colony affiliation. Colonies have exclusive memberships and colony residents promptly detect and reject non-members, i.e., non-nestmates. However, intruders, e.g., parasites, overcome chemical nestmate recognition and exploit colony resources. The chemical tricks that parasites use to circumvent host detection vary. Cleptoparasites have not enough recognition cues to allow prompt detection by their hosts; obligate social parasites use a mixture of lack of recognition cues and chemical mimicry; finally, facultative social parasites change colony odours by overmarking and may be particularly rich in epicuticular hydrocarbons. Each chemical strategy is deeply linked to the level of specialization and the quality of the host-parasite relationship.

Geographic differences in the chemical profiles of a social wasp

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Geographically close populations of the same species can be genetically more similar between each other than distant populations, as they may have been founded by colonists from the same source population and may have a higher gene flow. *Polistes biglumis* is a species of social wasps that has a patchy geographic distribution in mountains of South Europe. In some geographical areas populations are quite close, whereas in others they are more distant. As many social insects, *P. biglumis* wasps use cuticular hydrocarbons (CHCs) to discriminate between nestmates and non-nestmates. CHCs form the colony odor that varies largely among colonies, and this is important in order to prevent colony exploitation by conspecific and heterospecific intruders. In this study we investigated if the colony odor varies between populations as a function of their geographical distance. We studied 14 populations spread along western and central Italian Alps, for a total of 50 nests; we extracted CHCs from foundresses and analyzed their chemical composition using a gas-chromatograph. We found no significant correlation between chemical distances and geographical distances. This finding suggests that local conditions (among them biotic and abiotic factors) are more likely to influence variations in colony odor than geographic distance. Identifying these factors and disentangling their contribution in the differentiation of colony odor is one of the most stimulating challenges for future studies.

Insights into the complex biology of an invasive Drosophila pest, *Drosophila suzukii*

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Drosophila suzukii is a major pest in many Palaearctic areas, where females oviposit on ripening fruits to which larvae can cause severe damage. Initiating through morphological, behavioural, and electrophysiological studies, we sequenced the genome and transcriptome of *D. suzukii* with the two fold aims of assisting the different research lines and elucidating its evolutionary history. Comparative genomics were coupled with field observation to unveil the reasons behind its peculiar ecological behaviour. Our data support a late Miocene origin of *D. suzukii*, concomitant with events and conditions that suggest an adaptation to mountainous temperate forests, a hypothesis confirmed by our field trapping. Morphological studies included imaging different parts of male and female *D. suzukii* using Scanning Electron Microscopy (SEM) in comparison with *D. melanogaster* and *D. biarmipes*, sister species of *D. suzukii*. Olfactory structures distinguishing *D. suzukii* from its related species have been described. We tested the olfactory response of *D. suzukii* adults to its host plant volatiles using Y-tube olfactometer and the results show strong attractiveness by mated females. Electrophysiological experiments were performed using Gas-Chromatography Electro-Antennal-Detector (GC-EAD) for fruit volatiles collected from the Headspace and the profile of EAG-active volatiles has been identified using GC-MS. The future assays will be performed with the identified compound/s in single or in blend of synthetics using Single Sensillum Recording (SSR) with an aim of developing a semio-chemical which has a potential to be employed in environmentally-safe strategies for controlling the pest.

A social parasite exploits cues from the coevolutionary race between foodplant and host ant

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The choice of the ideal plant to ovipose on is an essential step for butterflies in order to ensure the survival of their offspring. The obligate myrmecophilous butterflies of the genus *Maculinea* represent an extreme case. Adult females have to select suitable food plants which are at the right phenological stage to ensure adequate nutrition to the larvae until the IV instar and situated in the proximity of *Myrmica* colonies to favour the encounter with the host ant. We studied the mechanism used by *Maculinea arion* females to identify food plants close to ant nests. In laboratory experiments we found that the presence of a *Myrmica* ant nest stimulates *Origanum* food plant to expand its bouquet of volatile substances. *Maculinea* females can follow some of these compounds to trace the adequate plant where to lay eggs, by exploiting the co-evolutionary ants-plant relationship. Here the interaction between plant (oregano) and insect (ants) occurs at a non-trophic level and opens a totally new field of research.

Oviposition-site selection in *Zerynthia polyxena* (Lepidoptera, Papilionidae) and its implication for conservation

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Selecting the oviposition site is a crucial event in butterflies’ life-cycle, especially for univoltine monophagous species having limited capacity of dispersal, such as those of the genus *Zerynthia*. This research investigates the oviposition behaviour and environmental factors influencing the egg density of *Zerynthia polyxena* on its food-plant, *Aristolochia rotunda*, at our “Capanne di Marcarolo” (NW Italy) study area.

Zerynthia polyxena is an endangered species, vulnerable by the effects of anthropic activities. It is included in the European “Habitats Directive” 92/43/EEC under Annex IV. In recent years, numerous extinctions of *Z. polyxena* have been recorded all across Europe, at population level. The main cause of extinction is found in habitat loss throughout the Italian territory, especially in the plains of northern Italy.

The presence and distribution of eggs on potential food-plants were recorded, while several other parameters, potentially affecting egg distribution, were collected and measured in the field.

Results are discussed in the light of potential implications for this species conservation and management, including its behaviour, ecology, morphology and molecular biology. We also tried to clarify the respective taxonomic status of *Z. p. polyxena* and its [sub-] species *Z. p. cassandra*. Nucleotide sequence variation analysis of mitochondrial regions was conducted, based at the moment only on Cytochrome C oxidase subunit and ITS sequences, to investigate supposed species-level distinction based on genitalia morphology.

These findings will be crucial for planning habitat restoration and/or population-restocking activities, aimed at the long-term conservation of this species.

Taxonomy, behaviour and life history of the endemic, slave-making ant *Myrmoxenus tamarae*

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Myrmoxenus is a particularly interesting genus of slave-making ants. *M. tamarae* is the only representative of this genus reported from the Caucasus. *M. tamarae* was described by K. Arnol’di in 1968, based on material collected by the Georgian myrmecologist Tamar Jijilashvili in 1963 from a nest of *Temnothorax* found near the village Daba in the Borjomi district (southern Georgia). Based on the brief original description and figures of *M. tamarae*, Buschinger (1989) concluded that *M. tamarae* might be a synonym of the widely distributed species *M. ravouxi*. Despite close morphological similarities to *M. ravouxi*, *M. tamarae* is still considered as a valid species endemic to Georgia and is listed by IUCN under category VU D2.

In December 2011, we analyzed the morphology, behavior, and life history of colonies of *M. tamarae* at the laboratory of Evolution, Behavior and Genetics at the University of Regensburg.

Seventeen characters were measured in 15 workers of *M. tamarae* and 24 workers of *M. ravouxi* from Austria, Georgia, Greece, France, Slovenia, and Turkey. In a discriminant analysis, workers of Turkish *M. ravouxi* and *M. tamarae* grouped together and were well-separated from all individuals of *M. ravouxi* collected in Europe.

Behavioral investigations showed big differences in activity between *M. tamarae* and *M. ravouxi*. *M. tamarae* is typically monogynous and appears to actively conduct slave-raids.

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Impact of habitat fragmentation on the diversity of Tachinidae (Diptera) inhabiting semi-natural grasslands

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The conversion of natural ecosystems for agricultural farming is one of the major threats to arthropod diversity. In this study, the parasitoid flies in the family Tachinidae (Diptera) were used as a model system to test the effects of habitat fragmentation on the third trophic level. A pan-trap sampling was conducted in 18 semi-natural grasslands along statistically orthogonal gradients of area, connectivity, and habitat heterogeneity. The sites were located in the province of Siena (Tuscany, Italy). The sampling was conducted every two weeks between March and November 2012 with a number of traps proportional to patch area. Three main swarming peaks were observed during the growing season. Species richness peaked in late spring, while the total abundance was highest in late summer. The most common species were associated to Hemiptera and Coleoptera hosts. Three species appeared to be dominant: *Zeuxia aberrans*, *Gastrolepta anthracina* and *Besseria reflexa*. Preliminary results indicate that species richness and abundance responded positively to habitat area while the effect of habitat connectivity was less clear. Within-patch habitat heterogeneity further appeared to be positively related to species richness. The work represents one of the few studies investigating the impact of habitat fragmentation on parasitoid communities. In particular, the results will help understanding the relative importance of habitat area and connectivity and altered habitat heterogeneity in driving the community response of this important parasitoid family.

Spatiotemporal dynamics and bio-ecology of sandflies in the region of Montpellier

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Sandflies are Diptera Nematocera of the Psychodidae family. Some species are described as vectors of leishmaniases, parasitic diseases caused by protozoa of the genus *Leishmania*. About 400 sandfly species exist in the Old World, 70 of them have been identified or suspected as vectors. In France, five species are present: *Phlebotomus ariasi*, *P. perniciosus*, *P. mascitii*, *P. papatas*i (Corsica) and *Sergentomyia minuta*. Visceral leishmaniasis caused by *Leishmania infantum* is endemic and transmitted by *P. ariasi* and *P. perniciosus* in the southern part of the country.

The objective of this work is to study how populations are spatially and temporally organized along a transect located in the region of "Le Vigan" where leishmaniasis is present. The abundance and distribution of species collected in 2010/2011 are confronted with the data collected 30 years ago. A geo-morphometric analysis performed on *P. ariasi*, the major species in our sample, provides information on the bio-ecology of these insects. Overall 3233 individuals were collected: 90.7% *P. ariasi*, 8% *S. minuta*, 1.2% *P. perniciosus*, and 0.1% *P. mascitii*.

This work shows that populations have decreased by 30% in 30 years. We found a structuring of the populations in this area according to the environment, temperature, humidity and altitude. For example, the density was more important in stations with sources of blood in abundance for *P. ariasi* and P. *perniciosus*, such as henhouses or kennels, and wilder areas for *S. minuta*. Morphometric analyses highlight a structuring in populations of *P. ariasi*, different in function of the sex.

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Exploring introduction pathways of alien xylophagous insects in Italy

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Invasive alien species cost to forestry billions of euros every year and the early detection of these species is becoming of utmost importance. The aim of this study is to improve the knowledge around the alien xylophagous species introduction patterns, understanding how the size of ports and the presence or absence of forest around the harbor can influence the arrival and the establishment of the alien wood-boring beetles (Scolytinae, Cerambycidae and Buprestidae) arriving at high-risk sites. From May to September 2012, thanks to the collaboration with national and local Plant Health Services, 15 Italian ports were monitored (Ancona, Bari, Cagliari, Catania, Genova, Monfalcone, Napoli, Porto Nogaro, Porto Torres, Palermo, Ravenna, Salerno, Trieste, Taranto, Venezia) setting up multi-funnel traps baited with attractive lures (ethanol, α-pinene, methyl-butenol, ipsenol and ipsdienol) both in ports (4 traps) and surrounding areas (3 traps), in order to analyze differences in alien and native insects trapped in ports of different size and with different habitat composition surrounding the port. Preliminary results (c. 50% of samples examined) indicate that the higher is the amount of imported commodities, the higher is the probability that an alien species can be introduced (5 alien species trapped in big ports and 2 in small ports). We also expect to catch a higher number of alien species in sites with a high proportion of forests surrounding the port, demonstrating the influence of habitat composition on the likelihood of alien species establishment.

Sampling and spatial distribution of phytoplasma vectors in the vineyard agro-ecosystem

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Sampling of phytoplasma vectors is crucial as it drives pest management, but the choice of methods depends on the kind of data to be obtained and the target species. Direct methods (sweep-net, vacuum devices, beating trays, counts, etc.) provide a sample at a single moment, are more time-consuming, but allow to collect live specimens; whereas indirect methods (colored sticky traps, malaise traps, etc.) provide samples along a window of time, but may be more selective for some species, or between genders. The optimal sample size may be defined by using sequential or binomial sampling plans based on crowding indexes (Taylor's Power Law, Iwao's Patchiness, etc.). Estimation of vectors at un-sampled locations may be obtained via spatial interpolation e.g. IDW, kriging etc., or Artificial Neural Networks (ANNs), whereas dispersal can be studied with mark-release-recapture (MRR), or mark-capture (MC) techniques, using fluorescent dusts, or proteins. In the vineyard agro-ecosystem, two vectors may be used as an example for different sampling methods: *Scaphoideus titanus* (Hemiptera, Cicadellidae), a grapevine specialist; and *Hyalesthes obsoletus* (Hemiptera, Cixiidae), that lives on weeds (the nymphs feed on the roots), and only occasionally on grapevines. Adult *S. titanus* are easily collected with yellow sticky cards, whereas nymphs can be counted directly on leaves applying a sequential sampling plan. These methods may not be applied for *H. obsoletus*, as counts of nymphs on the roots would be too time consuming, and adults are not strong fliers and are more easily collected with a sweep-net.