[P2.06]

Nicotine and clothianidin effects are differently modulated by PKC-pathways through alpha-bungarotoxin-insensitive nicotinic receptors of insect neurosecretory cells

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Nicotinic acetylcholine receptors (nAChRs) are expressed in the insect central nervous system and they are the main target of neonicotinoid insecticides. The agonist activity of neonicotinoids is well studied, and it was demonstrated that they act preferentially as full or partial agonists of insect nAChRs. Two alpha-bungarotoxin (α-Bgt)-insensitive nAChRs, named nAChR1 and nAChR2, were identified in the cockroach *Periplaneta americana*. They differed in their pharmacology and were regulated by distinct intracellular messengers. nAChR1 was sensitive to imidacloprid, inhibited by d-tubocurarine and up- and down-regulated by two protein kinases C (PKCs), respectively PKC-1 (calcium-sensitive) and PKC-2 (calcium insensitive). nAChR2 was insensitive to imidacloprid, inhibited by mecamylamine and sensitive to intracellular and extracellular calcium. Here, we further analysed the involvement of intracellular pathways in the sensitivity of nAChR1 and nAChR2 to neonicotinoids. Because imidacloprid did not affect nAChR2, we studied the effects of 1,2 dioctanoyl-*sn*-glycerol (DiC8), a diacylglycerol analogue known to activate PKC-2, on the response of the two α-Bgt-insensitive receptors to nicotine and clothianidin. Our results indicated that the effects of nicotine and clothianidin on both nAChR1 and nAChR2 were differently modulated by PKC pathways. Indeed, nicotine effects required the activation of PKC-1 and PKC-2, whereas clothianidin effects occurred in a calcium-independent manner, suggesting that they were modulated by PKC-2. In final, our data demonstrated that the intracellular modulation of nAChRs is complex and a better understanding of its underlying mechanisms could lead to new strategies in pest insect and disease vector control.

Keywords: Insect nicotinic acetylcholine receptors, DUM neurons, Calcium, Protein kinases C

[P2.07]

Identification of cis-regulatory region controlling semaphorin-1a expression in the *drosophila* embryonic nervous system

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The *Drosophila* transmembrane semaphorin Sema-1a mediates forward and reverse signaling that play an essential role in motor axon pathfinding during embryonic neural development. Previous immunohistochemical analysis revealed that Sema-1a is expressed on most commissural and longitudinal axons in the central nervous system (CNS), and five motor nerve branches-ISN, ISNb, ISNd, SNa, and SNc-in the peripheral nervous system (PNS). However, Sema-1a-mediated axon guidance function contributes robustly to both ISNb and SNa, and slightly to ISNd and SNc, but not to ISN motor axon pathfinding. To identify *cis*-regulatory elements (CREs) that are required to direct Sema-1a expression during embryonic neural development, we took advantage of a collection of GAL4 lines driven by flanking and intronic DNA fragments of *Sema-1a*. We crossed each line to a *UAS-TaumycGFP* reporter line and then stained resulting embryos with anti-myc or anti-GFP antibodies to visualize the expression pattern of each GAL4 line. Here, we uncover two CREs that drive reporter expression on both ISNb and SNa nerve branches. Through complementation test with a *Sema-1a* loss-of-function mutant, we found that neuronal expression of Sema-1a driven by either CRE restore robustly the CNS and PNS motor axon guidance defects observed in *Sema-1a* homozygous mutants. Ongoing experiments will address the expression pattern of Sema-1a at neuromuscular junction during larval stages. This issue is related to a question of whether Sema-1a plays a role in synaptogenesis at the *Drosophila* neuromuscular junction.

Keywords: cis-regulatory Element, Semaphorin-1a, Axon Guidance

[P2.08]

Understanding the molecular and neural basis of olfaction in red palm weevil using gene silencing and odor-evoked brain activity studies.

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The red palm weevil (RPW) *Rhynchophorus ferrugineus* (Olivier), (Coleoptera: Curculionidae) is one of the most devastating pest of palm trees around the world. Pheromone communication, mediated mainly by an aggregation pheromone composed of 4-methyl-5-nonanol and 4-methyl-5-nonanone (9:1) is essential for mating, survival and aggregation of these insects. Thus, understanding the molecular and neural basis of olfaction is important for developing effective pest control strategies against RPW. We have recently elucidated the molecular components of peripheral olfactory signaling in RPW by finding the key odorant receptors (ORs) and odorant binding proteins (OBPs) specific to major aggregation pheromone component *ferrugineol*, by using RNAi based screening of RPW transcriptome. To further evaluate the olfactory signaling in the brain, and to characterize the receptors, we combined gene silencing and calcium imaging techniques in the present research. We studied the RPW neuroanatomy and then compared the odor-evoked brain responses in OR-silenced (*RferOR1*) and control insects, using the aggregation pheromone components and a kairomone, ethyl acetate. The findings provide the antennal lobe morphology and present an initial attempt to measure the odor-evoked brain responses to understand the olfactory signal processing in the weevil. This research and findings could serve as a platform for further studies on elucidating the complete olfactory coding in this important pest of palm trees, enabling better pest control strategies like developing biosensors for early pest detection.

Keywords: Neuroanatomy, Olfactory signaling, Red palm weevil, Odorant receptor

[P2.09]

A study on habitats and behavioral characteristics of hornet wasp (Hymenoptera: Vespidae: *Vespa orientalis*), An important medical-health pest

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Introduction: Hornet wasp or *Vespa orietalis* is one of the social and stinging insects of Iran that makes its nest mostly in the holes and cracks in the ground. Given the point that no study has been conducted in this case, yet, in this study, the nests of this arthropod are studied in four mountainous and plain areas in Kashan .

Methods: Present study was of descriptive type and during it, four nests of *V. orientalis* in four agricultural, residential, administrative and mountainous regions were identified and studied. The identification of the nests of these animals was done on the basis of observation abundance of this wasp around the nests, defense and the existence of definite tumulus in farmlands from May to September 2016-2-17.

Results: Tumulus, the nest of *V. orientalis* was irregular in farmland and imperceptible in residential and mountainous regions. The entrance of the nests in the soil was 3×5 cm. The nests of this hunter wasp were in the regions where a large number of its feeding arthropods were active. This study showed that the red wasps feed on cockroaches in residential regions, Schirasi woodlouse (*Hemilepistus schirasi*) and the larvae of butterflies in agricultural lands, and honey bees, larvae of butterflies and other insects in other regions. The results of this study revealed that the nests of *V. orientalis* as an opportunistic arthropod are seen in different places and if needed , this animal cleans the nests and to some extent it is a builder, occupier or tenant of the nest.

Conclusion: The existence of this animal that nests in human habitats causes danger especially for children.

Keywords: Hornet Wasp, Vespa Orientalis, Behaviour, Ecology

[P2.10]

Olfactory receptors essential for the blood-feeding process of the major disease vector, *Aedes aegypti*

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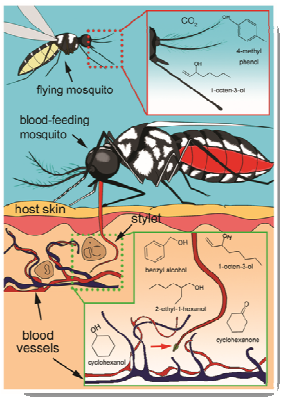
The yellow fever mosquito, *Aedes aegypti* (*Ae. aegypti*) is a major disease vector for dengue virus, Zika virus, yellow fever and chikungunya. One of the poorly understood aspects of mosquito blood-feeding behaviours is how they target an optimal site in order to penetrate the skin and blood vessels without alerting the host animal. Here we provide new findings that the piercing-sucking stylet of Ae. *aegypti* is an essential apparatus for the final stage in blood feeding behaviour. Indeed, the stylet possesses olfactory receptor neurons that express two conventional olfactory receptors of *Ae. aegypti* (AaOrs), AaOr8 and AaOr49, together with the olfactory co-receptor (AaOrco). In vitrocalcium imaging using transfected cell lines demonstrated that AaOr8 and AaOr49 were activated by volatile compounds present in blood. Gene expression inhibition of these Ors interferes with blood-feeding behaviours. In silico protein modeling and mutaganasis also demonstrated structural interactions between these Ors and ligands. Taken together, we identified olfactory receptor neurons in the stylet involved in mosquito blood feeding behaviors, which in turn indicates that olfactory perception in the stylet is necessary and sufficient for mosquitoes to find host blood in order to rapidly acquire blood meals from a host animal.



Keywords: Mosquitoes, Aedes aegypti, Olfaction, Olafactory receptor

[P2.11]

Mechanism of acetic acid avoidance in *Drosophila*

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Abstract

Sour taste allows for the detection of hydrogen ions and organic acids. It is one of the five basic tastes, and along with other chemical and textural features, allows animals from flies to humans to discriminate between foods that are safe and appealing from other options that are dangerous. While low levels of certain acids are attractive, high levels are repulsive. The aversive makes sense, as foods that are very acidic may be spoiled due to excessive microbial growth, and if consumed, can lead to adverse effects.

Here I will present the possibility that each organic acid is sensed by different receptor. Such a narrowly tuned taste receptor might be important for insects to survive in nature.

Introduction

Studies on taste modality using the animal model *Drosophila melanogaster* have clarified a number of uncharacterized mechanisms of sensory responses. Gustatory receptors that are expressed in taste organs are responsible for the acceptance and rejection of different foods. Food preference largely depends on taste and smell, as well as vision. The gustatory organs in flies include the labellum, legs and internal organs. We screened possible candidates including gustatory receptors and ionotropic receptors for different acid taste.

Methods

We used behaviour, electrophysiology and immunohistochemistry.

Results

We identified one ionotropic receptor, which is narrowly tuned by acetic acid, but not other organic acids. Furthermore, this avoidance sense is mediated by 11 bitter-sensing GRNs.

Discussion

This is first report in taste that sour is not a simple response from hydrogen ion. Humans also can sense different flavour by sensing different organic acids. I would raise the possibility that insects have a largely developed repertoire to sense many different kinds of acids on food, including fungi metabolites.

Keywords: Drosophila, taste, sour, ionotropic receptor

[P2.12]

Discovering the umami receptor in the Western honey bee, *Apis mellifera*

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Taste is responsible for choosing profitable food sources and for nest mate recognition in honeybees. Taste detection for food sources occurs within cuticular hairs located on the antennae, on the mouthparts, and on the tarsi of the forelegs. Among food sources, umami taste perception indicates the presence of amino acids, which are essential nutrients. Although the physiology of umami perception has been described in mammals, how insects detect amino acids remains unknown. We functionally characterized a gustatory receptor responding to L-amino acids in the western honey bee, *Apis mellifera*. Using a calcium-imaging assay and two-voltage clamp recording, we found that one of the honey bee's gustatory receptors, AmGr10, functions as a broadly tuned amino acid receptor responding to glutamate, aspartate, asparagine, arginine, lysine, and glutamine, but not to other sweet or bitter compounds. Furthermore, the sensitivity of AmGr10 to these L-amino acids was dramatically enhanced by inosine-5'-monophosphate (IMP). Contact sensory hairs in the mouthpart of the honey bee responded strongly to glutamate and aspartate, which house gustatory receptor neurons expressing AmGr10. This functional organization of the umami receptor of the honey bee strongly indicates the correlation of internal and external sensing of amino acids. Interestingly, AmGr10 protein is highly conserved among hymenoptera but not other insects, implying unique functions in social insects.

Keywords: Gustatory receptor, Umami receptor, Honey bee, Apis mellifera

[P2.13]

Enhanced gene expression of enzymes involved in dopamine biosynthesis by a juvenile hormone analog in male honey bees

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Biogenic amines in the central nervous system can be regulated by hormonal effects. In honey bee males, both dopamine (DA) and octopamine (OA) in the brains increase with age until 8 days old, together with the titer of juvenile hormone (JH) in hemolymph. To clarify the mechanisms underlying the age-dependent increases of DA and OA in the brains in the males, the possibility that JH up-regulates the gene expression of enzymes involved in the biosynthesis of DA and OA in the brains of males was tested.

We initially examined age-dependent enzymatic activities and gene expression of enzymes for DA and OA biosynthesis, and then quantified the gene expression levels of enzymes in response to a JH analog application (treated concentration: 10 or 100 μg).

The enzymatic activities for DA biosynthesis in the brains were significantly higher in 4- and 8-day-old males than in 0-day-old males. Relative gene expression levels of tyrosine hydroxylase gene (*Amth*) for DA biosynthesis in the brains were significantly higher in 4-day-old males than 0- and 8-day-old males, whereas those of DOPA decarboxylase gene (*Amddc*) for DA biosynthesis were higher in 4- and 8-day-old males than 0-day-old males, and tyrosine decarboxylase gene (*Amtdc*) and tyramine-β-hydroxylase gene (*Amtbh*) for OA biosynthesis were significantly higher in 8-day-old males than in 0- and 4-day-old males. The application of a JH analog to 0-day-old males for two days enhanced the gene expressions of *Amth* and *Amddc*, but not *Amtdc* and *Amtbh*.

Our results suggest that quantitative changes in the gene expression of the enzymes are related to an increase in the amount of DA and OA in brains and support the possibility of the up-regulation of enzyme gene expressions for DA biosynthesis by JH in male honey bees.

Keywords: Biogenic amines, Brain, Sexual maturation, Social insect

[P2.14]

Genetic variability of a winter-emerging Chironomidae in trout streams of southeastern Minnesota

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Groundwater inputs to trout streams in the Driftless Region of Southeastern Minnesota average 8° C year round, keeping streams cool in summer, but ice-free in winter. As downstream distance from groundwater inputs increases, many of the streams freeze over, creating a mosaic of isolated, open-water areas from which *Diamesa mendotae* (Muttkowski) emerge in winter. This species is highly cold-adapted, emerges at sub-freezing air temperatures, is long lived as an adult, with super cooling points of ~ -20° C, successfully mates on snow banks adjacent to streams, but does not emerge during warmer months. It is presumed that minimal gene flow occurs among populations, and we are testing the hypothesis that selection has favored a narrow range of genetic variability that allows for specialized physiology and behavior suited for survival of the harsh conditions into which adults emerge. An alternative hypothesis is that selective forces related to differing water quality and/or habitat conditions near areas of groundwater input operate primarily on the larval portion of the life cycle and produce population-specific genetic compositions that vary across streams. To test the alternatives, we sequenced a 581 bp mtCOI fragment of specimens from two populations in different streams and observed 10 haplotypes. Maximum uncorrected pairwise distance observed between haplotypes was 0.87% within one population, 1.04% within the second population, and 1.57% between haplotypes among populations. Consequently, neither alternative hypothesis appears to be strongly supported. The number of haplotypes found appears high but is similar to three Holarctic species of *Chironomus* (Martin *et al.*, 2002). Preliminary data obtained to date do not allow in-depth analysis, but the mtCOI sequence data appear sufficiently variable to detect intraspecific population structure in *D. mendotae*. Specimens collected this winter from seven additional streams are currently being assessed and results will be integrated into this poster.

Keywords: Chironomidae, Winter-emerging, genetic, stenothermic

[P2.15]

Comparative transcriptomes of larval fat body in *Helicoverpa assulta* under different temperature conditions

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Insects are known to live at wide range of temperature in terrestrial environments, but they can't survive when exposed to over 40□ or below supercooling point. To identify genes affected by temperature, we analysed the transcriptomes of fat body under the different conditions. The larvae of *Helicoverpa assulta* have been reared at high (35□), low (3 to 10□), and room temperature (25□; control). Genes such as cuticular proteins, fatty acyl □9 desaturase and glycerol 3 phosphate dehydrogenase were up-regulated whereas chitin synthase, catalase, and UDP-glycosyltransferase were down-regulated at low temperature. In contrast, superoxide dismutase, metallothionein 2, phosphoenolpyruvate carboxykinase and trehalose transporter have been up-regulated at high temperature. In addition, expression of heat shock protein and glutathione peroxidase was increased at high temperature, but decreased at low temperature. These results suggests that these genes can be available as molecular markers for climate change of insect pests in agriculture.

Keywords: Climate change, Temperature, Transcriptome, Marker

[P2.16]

Biological response to chlorpyriphos of two alpine chironomid species (*Diamesa* spp.)

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Freshwater organisms at high altitudes are exposed to a double threat, climate change and associated glacier retreating, and exposure to chemical pollution transported to the glaciers by the atmosphere. Organophosphorus pesticides, among which the insecticide chlorpyrifos (CPF), are extensively used in agriculture in the last twenty years, even in alpine valleys. It was found during the ice-melt period in the glacier-fed strem Presena, fed by the homonimous glacier in the Trentino Province (Italian Alps), with an environmental concentration ranging from 6.13 to 6.83 ng/L. The effects of CPF were evaluated at sub-organismal level on two chironomid species, *Diamesa zernyi* and *Diamesa steinboecki*, both collected in the Presena stream (at 2685 m a.s.l.), in summer 2018. The two species are known to be cold stenothermal, with *D. steinboecki* restricted to kryal habitats. Larvae were exposed to three sub-lethal concentrations corresponding to LC10 (1.1 μg/L), 1/10 LC5 (0.524 μg/L) and 1/10 LC10 (0.11 μg/L) for 72 h, at the environmental temperature (2 °C), without food, with aeration. Two endpoints were evaluated: a) in both species, gene expression by Real-Time PCR of four candidate genes known to be involved in chemical stress response and detoxification mechanisms (two forms of *hsp7*0, *cyp450 and hsc70*); b) in *D. zernyi*, the oxidative stress by malonaldehyde (MDA) and Protein carbonyl (PCC) test. The results showed that *D. zernyi* is more sensitive to the CPF than *D. steinboecki*, due to the alterated gene expression of three genes (*hsp70*, *cyp450* and *hsc70*) observed in *D.zernyi*. The second endpoint highlighted as CPF caused oxidative stress at sub-lethal concentrations, as both lipid peroxidation and protein carbonilation. Overall these findings suggest that wild populations of glacier-fed streams are under physiological stress by chemicals.

Keywords: climate change, Chlorpyrifos, Diamesa, sublethal concentrations

[P2.17]

Investigation on the ice fly *Diamesa steinboecki* thermal tolerance with a molecular approach

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The ice fly *Diamesa steinboecki* Goetghebuer, 1933 is a cold stenothermal non-biting midge colonizing only glacier-fed streams in the East Palaearctic region. It completes the life cycle at water temperature <4 °C. Long-term series of ecological data highlighted that glacier retreating under climate change is affecting its distribution in the Alps, due to the alteration of the habitat conditions in which it lives. Scarce information is available on thermaltolernace of other *Diamesa* species living in cold freshwaters (e.g., *Diamesa cinerella* Meigen, 1835), highlighting their capacity to develop an Heat Shock Response (HSR) under warming at temperatures >26 °C. To investigate the thermotolerance in the ice fly, IV-instar larvae of *D. steinboecki* collected in two glacier-fed streams in the Italian Alps (Presena and Mandrone, ≈2600 m a.s.l., Trentino) were exposed to increasing temperature starting from 26 °C for 1h followed by 1h of recovery at 2 °C. An extraordinary high lethal temperature was estimated (LT_{50}= 39.2 °C, LT_{99}= 40,3 °C). Using the same exposure protocol at 26, 28, 30, 32, 34, 36 and 38 ºC, gene expression analysis of four candidate genes involved in stress response was performed (*Cyp450*, two forms of *hsp70* and *hsc70*) by retrotranscription and Real-Time PCR. The results suggest that its strong short-term thermotolernce is associable to an HSR, with a significant espression alteration of the heat shock genes *hsp70* and *hsc70*.Further research is needed to evaluate the resistance to prolonged exposure to tempertaures higher that the natural one, giving new insights on the biological response to climate change of alpine species threatened by exctinction.

Keywords: Diamesa steinboecki, climate change, thermal tolerance

[P2.18]

Hologenome profiling of Frakliniella occidentalis reveals a complex microbiome and confirms the presence of two novel symbionts

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Frankliniella occidentalis (Pergande), common name Western Flower Thrips, is a global agricultural pest due to its high reproducibility and dispersal rate, extreme polyphagy and propensity to develop pesticide resistance. Despite this, only one F. occidentalis draft genome is available and the study of its microbiome has been limited to culture based techniques. Thus only two symbionts (Bfo1 and Bfo2) have been identified in this insect and their genomes sequenced.

We have applied deep parallel sequencing to total DNA isolated from specimens of various F. occidentalis populations in order to visualise their hologenome (host and microbiome genomes). Short sequence reads were classified using Kraken with a custom database composed of the available F. occidentalis genome and all genomes from Bacteria, Archaea, Plantae and Fungi in NCBI. This analysis assigns a taxonomic classification to individual short reads, generating a specific and quantitative microbiome profile. Our observations reveal a complex and partially conserved microbiome associated to F. occidentalis and confirming the predominant presence of Bfo1 and Bfo2. We believe that the microbial diversity observed may contribute to the high invasiveness and adaptability of F. occidentalis.

Two new symbionts of the genus Acinetobacter were cultured from F. occidentalis specimens. The association of these bacteria to the host was confirmed by analysis of the hologenome profiles. The methodology described here demonstrates that hologenome visualisation is a more accurate tool to describe and study the microbiome associated to insect species, in contrast to traditional 16S rRNA-based metagenomic approaches.

Keywords: Frankliniella occidentalis, hologenome, symbiont, genomics

[P2.19]

Gut microbiota of field-collected and laboratory-selected insecticide resistant larvae of *Spodoptera frugiperda* and the diversity of insecticide degrading bacteria

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Bacterial symbiosis directly affects insect physiology and development, facilitating insect adaption to new environmental conditions. Insects and associated microbiota acquire phenotypic features that guarantee survival against biotic and abiotic stress factors, as in situations of food shortage and exposure to toxic compounds. Using *Spodoptera frugiperda* (Lepidoptera: Noctuidae) and its associated gut microbiota as a model, we aimed to evaluate the influence of selection pressures on the structure, diversity and fitness of the gut microbiota. We focused our work on insecticide resistant phenotypes of *S. frugiperda* using insecticides as a source of selection pressure. We thus compared the gut microbiota composition of susceptible and insecticide-resistant strains of *S. frugiperda*, as well as the microbiota of natural populations sampled in the field. The experiments were conducted through metagenomic analysis of the 16S ribosomal (16S rRNA) regions v3 and v4, isolation and culture of bacteria in selective minimal medium (SMM), and analysis of the growth of isolates in SMM using multiple insecticides as the only source of carbon. The degree of insecticide exposure influenced the composition of the gut microbiota of *S. frugiperda*, as well as the growth capacity and potential of its members to use the insecticides tested as a source of carbon. In addition, most of the phylotypes isolated in SMM were fixed in the gut microbiota of natural populations of *S. frugiperda*, indicating the importance of the symbiotic relationships identified and their possible role in the detoxification of insecticides in the host.

Keywords: Insecticide resistance, Fall armyworn, Insecticide degradation, Sustainable pest management

[P2.20]

Presence of bacterial endosymbionts in mites of economic importance in Spanish citrus orchards

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Introduction: Endosymbiotic bacteria can have a large impact on their hosts. In arthropods they have been described to modify their reproductive parameters, defense mechanisms, resistance to different types of stress, etc. In this study we have determined the incidence of four endosymbiont species (belonging to the genera *Cardinium*, *Rickettsia*, *Spiroplasma* and *Wolbachia*) in different Spanish populations of the citrus mite *Tetranychus urticae* Koch (Acari: Tetranychidae), one of the main pests of this crop, as a previous step to characterize their effect on it. Furthermore, we have also analyzed other species that share crop and/or habitat as the Tetranychidae *Panonychus citri* (McGregor), *Aplonobia histricina* (Berlese), *Eutetranychus banksi* (McGregor), *Eutetranychus orientalis* (Klein), *Tetranychus evansi* Baker and Pritchard and *Tetranychus turkestani* Ugarov and Nikolskii and the Tarsonemidae *Poliphagotarsonemus latus* (Banks).

Methods: Mite samples were analyzed by PCR first with 16S universal primers and after with specific primers of the genera *Wolbachia*, *Cardinium*, *Rickettsia* and *Spiroplasma*. Afterwards, a multilocus phylogenetic approach using 16S, *FtsZ* and *wsp* regions was done to determine the *Wolbachia* diversity in *T. urticae* citrus populations. A phylogenetic reconstruction of *Wolbachia* and *Spiroplasma* using the homologous 16S region was done for all the tetranychidae species included in this study.

Results and discussion: Spanish citrus mites showed a variable infection rate. *Wolbachia* spp was the predominant endosymbiont in all species analyzed except for *P. citri* and *E. banksi* that no *Wolbachia* was found. The other bacterial species appeared with a variable incidence, even as a double infection. The relationship between *Wolbachia* and other endosymbiont presence and host plant specialization of citrus mite populations deserves further research. This knowledge opens new opportunities to control mite pests in citrus.

Keywords: Wolbachia, Spiroplasma, Cardinium, Tetranychidae

[P2.21]

Differential mating behaviours of a tetracycline-treated *Tetranychus urticae* laboratory strain and their effect on mite microbiota

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Introduction: The two-spotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae) is an important pest of citrus in Spain. The obligate intracellular bacteria *Wolbachia* spp has been described to infect this mite, which can also harbor other endosymbionts as *Cardinium* and *Spiroplasma*. Facultative symbionts as *Wolbachia* are mutualistic in the context of various ecological interactions, and they can have important implications for the management of pest species. For example, *Wolbachia* can cause several types of reproductive disorders in its host like male feminization, thelytokous parthenogenesis, cytoplasmic incompatibility, or male death. In *Tetranychus* genera. *Wolbachia* has been described to induce, to some extent, cytoplasmic incompatibility, depending on the mite species and/or the population origin. Therefore, the aim of this study was to elucidate if *Wolbachia* manipulates the reproduction of the Spanish population of *T. urticae*. A tetracycline treatment was used.

Methods: The effect of tetracycline treatment on the spider mite reproduction was evaluated between T- and U- mites (treated and untreated mites, respectively). Four different crosses were tested: 1) U-♀ x U-♂; 2) T-♀ x T-♂**;** 3) U-♀ x T-♂; 4) T-♀ x U-♂; with two controls: 1) virgin untreated females (U-♀) and 2) virgin treated females (T-♀). Antibiotic treatment effect in mite microbiota a 16S deep sequencing was performed using two different combinations of primers. The sequences obtained were compared with two databases for OTUs identification: (1) SILVA and (2) a non-redundant nucleotide database.

Results and discussion: The Tetracycline treatment produced cytoplasmic incompatibility in *T. urticae* by affecting offspring sex ratio, indicating the presence of a mating distorter bacterium in our *T. urticae* population. The 16S deep sequencing revealed that the antibiotic treatment removed mainly *Wolbachia* spp., which could be the responsible of the cytoplasmic incompatibility observed. Bacteria diversity identification was affected by the selected primers and by the database used.

Keywords: Wolbachia, Citrus, Cytoplasmic Incompatibility

[P2.22]

Two complete mitochondrial genomes of the invasive species, *Metcalfa pruinosa* (Hemiptera: Flatidae): genomic comparison among species of Fulgoroidea and selection of variable sites useful for population genetic analysis

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The flatid planthopper, *Metcalfa pruinosa* (Hemiptera: Flatidae), which is an international invasive species, was detected in Korea, and now is widespread abundantly in Korea. A previous studies depicting origin and sequence variability of the species using DNA barcoding region with the samples collected most abundantly from Korea showed relatively low sequence variability (3 haplotypes, 3 variable sites, and 0.636% of maximum sequence divergence). Thus, additional markers that reveal higher variability were necessitated to scrutinize population structure in connection with dispersal and invasive dynamics among international populations. Therefore, we sequenced two complete mitochondrial genomes (mitogenomes) of *M. pruinosa*, from the two haplotypes occurring in Korea (H1 and H3). Comparison of the two mitogenomes each with 16,312 and 16,314 bp evidenced that one region located in the A+T-rich region to provide higher number of haplotypes (4 vs. 3), sequence divergence (1.636% vs. 0.636%), and variable sites (7 vs. 3) than those of DNA barcoding region from the screening test using 13 representative individuals. This variable region, in concatenation with the currently available DNA barcoding region might be useful for populations genetic analysis of worldwide populations including those of Korea.

Keywords: Invasive species, DNA barcode, Mitochondrial genome, Population genetics

[P2.23]

Microbial dynamics of Xyleborus affinis during its cycle life

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Xyleborus affinis Eichhoff (Coleoptera: Curculionidae: Scolytinae) is an ambrosia beetle with a pan-tropical distribution in more than 26 woody hosts. This species belongs to fungus-farming lineage, characterized by cultivating symbiotic fungi with which it establishes a mutualistic nutritional relationship. Although it is possible to rear *X. affinis* in the laboratory with life cycle of 30 to 35 days, there is little knowledge regarding the role played by microbial community and their dynamics during the development of this species. We analysed the bacterial and fungal microbiome of the galleries, foundresses and brood during the life cycle of *X. affinis,* using a metagenomics approach. The results showed that the microbiome populations consist of 34 fungal and 428 bacterial OTUs. The different bacterial and fungal populations exhibited dynamics that depended on the origin of the sample and the sampling time. In addition, analyses of the metabolic capabilities revealed a role of the bacteria microbiome in the preparation of the niche for fungal growth, helping in the degradation of the fungal and plant cell wall and fixing of atmospheric nitrogen. This is the first study analysing the microbial populations associated with ambrosia beetles by a metagenomic approach and predicting the metabolic role of the bacterial microbiome.

Keywords: Ambrosia beetles, Microbiome, Metagenomics

[P2.24]

DNA barcodes of ants

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The family Formicidae includes some of the major agricultural pests. One factor that influences agricultural pest management has been the lack of taxonomic knowledge. The study aims to examine sequence divergence in mitochondrial *cytochrome c* oxidase I (COI) sequences among ant taxa and examine the efficiency of mitochondrial DNA barcodes in species identification together with the morphology-based approach. Six hundred twenty-four (624) specimens were analyzed using the mitochondrial cytochrome c-oxidase I. Seventy-seven percent were successfully recovered and submitted in an online genomic database, Barcode of Life Data System (BOLD). CO1 divergences between congeneric species averaged 13.49% (range 1.21 – 50%), whereas those for conspecific individuals averaged 0.26% (range 0.0 – 2.89%). The result showed close sequence matches at less than 3% divergence threshold were detected for 27 species from the Mindanao faunal region, Philippines. In addition, DNA barcode data have shown to be effective in discriminating among the sampled ant specimens and contributed to the detection of probably new record of Philipppine ants, the *Colobopsis cylindrica* and *Leptogenys kraepelini*, both with matching similarity of 100% in BOLD and Genbank databases.

Keywords: Cytochrome c oxidase 1, Divergence threshold, Mitochondrial gene, Sequence divergence

[P2.25]

The role of gene flow in connecting populations of endangered skipper butterflies in the United States: using genetic data to inform management strategies

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Biodiversity loss (at species and genetic scales) has been exacerbated in recent decades due to habitat fragmentation. Species organized in metapopulations may experience increased risk from fragmentation as their persistence relies on a delicate balance of gene flow, maintenance of genetic diversity, and local adaptation. Landscape modifications that modify gene flow pose risks of further endangering metapopulation fragments and perpetuating an extinction vortex even when overall genetic diversity remains high. Butterflies are charismatic microfauna that offer an excellent opportunity to evaluate the consequences of how fragmentation disrupts gene flow between population segments. In this study, we use the Florida duskywing butterfly *Ephyriades brunnea* (Lepidoptera: Hesperiidae) to evaluate how connectivity between remnant pine rockland habitat fragments in South Florida is effected by habitat loss and fragmentation. We hypothesize that certain populations hold unique genetic diversity and that human alteration of the landscape promotes asymmetrical gene flow and disrupts connectivity between remaining fragments. We non-lethally sampled single legs from populations on both mainland and Florida Keys locations. Using PacBio next-generation sequencing, we sequenced a voucher specimen of *E. brunnea* and generated thousands of microsatellite markers. After extensive marker screening with an M13-tag approach, we genotyped all templates using 15 fluorescently-tagged microsatellite markers to assess genetic diversity and range-wide population structure. Highly-polymorphic markers allowed for fine-scale spatial differentiation and identification of source-sink dynamics and asymmetrical gene flow in a molecular ecology context using descriptive and Bayesian statistics. We identified populations that retain unique genetic diversity (i.e. private alleles and unique allele frequencies) that may warrant additional protection measures. We also identified disconnected fragments that may be at risk of local extirpation due to stochastic events. Given the multiple threats facing this species, including increased hurricane activity, we consider restoring connectivity and maintaining specific populations to enhance the security of this species.

Keywords: microsatellites, conservation genetics, population genetics, biodiversity

[P2.26]

A preliminary molecular phylogeny shows Japanese and European populations of the red mite *Balaustium murorum* (Acari: Trombidiformes: Erythraeidae) to be closely related

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The red mite *Balaustium murorum* (Hermann) inhabits the Western Palaearctic realm and is well adapted to man-made structures. In Japan, *B. murorum* had been reported more frequently after the 1980s. A molecular phylogeny based on the nuclear 18S rRNA and mitochondrial COI genes, and including *B. murorum* individuals from Japan, Germany, France, Austria and Poland, and representatives of related species from Japan showed four *Balaustium* species-level lineages in Japan (*B. murorum*, *Balaustium* sp. 1, *Balaustium* sp. 2, *Balaustium* sp. 3). The *B. murorum* lineage shared identical 18S sequence and COI haplotype with the European population. *Balaustium* sp. 1 was detected from the Tokyo and Misaki area (Honshu Island) and was the sister group to *B. murorum*; the other two lineages inhabited coastal environments of Erimo, Hokkaido Island (*Balaustium* sp. 2) and Ainan, Shikoku Island (*Balaustium* sp. 3). The high genetic distances among these four lineages indicate that each lineage is a distinct species, with three of the lineages representing undescribed species. Our results are compatible with the conclusion that *B. murorum* was introduced to Japan from Europe, although our study did not resolve the polarity or timing of migration events.

Keywords: Pest, Invasive species, COI

[P2.27]

Fighting the olive fruit fly *Bactrocera oleae* by hitting its bacterial symbionts: investigating *Erwinia dacicola* -host interactions

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The olive fruit fly *Bactrocera oleae* causes huge losses in olive production. The fly deposits its egg in the olive and the larvae develops within the fruit, causing destruction of the pulp and premature ripening. Control of the fly is largely dependent on the use of insecticides, but this is hampered by the development of resistance. The development of new environmentally friendly methods is of high priority. When the fruit fly larva develops in the green unripe olives the presence of the symbiotic bacterium *Erwinia dacicola* is absolutely required and thus can be considered a possible target for intervention. This rod-shaped Gram negative bacterium is closely related to free-living *Erwinia spp*. The study of this bacterium is restricted by the fact that the bacterium cannot be cultivated outside the fly. Furthermore, laboratory strains of *B. oleae* well adapted to artificial food are devoid of the bacterium. We have developed a robust method to transfer *E. dacicola* from wild caught flies to a laboratory strain of *B. oleae* and we show that the transferred bacteria are stably transmitted for at least six generations allowing studies of the interaction of host and symbiont in a controlled environment. We have also investigated the number of *Erwinia* in different tissues and life stages of fly populations originating from green and black olives, by employing advanced confocal microscopy approaches and qPCR. Furthermore, we will present primary analyses of genomic and transcriptomic data focusing on genes putatively involved in tissue specific host-symbiont interaction. Fighting olive fruit fly by eliminating its bacterial symbionts, by classical and/or biotechnology based approaches, could represent a valuable alternative pest control option.

Keywords: olives, olive fruit fly, bacteria, Erwinia dacicola

[P2.28]

Preliminary results on microbiome of *Frankliniella occidentalis* (Thysanoptera, Thripidae) natural populations in Southern Italy

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Since its introduction into Europe, at the end of 1980s through the horticultural trade of living plants, *Frankliniella occidentalis* (Pergande) (Thysanoptera, Thripidae) has become one of the most harmful pest to economic importance crops, in greenhouses and in open field.

This highly polyphagous species infests seed, flowering, nursery, strawberry, peach, grape and cotton crops and the symptoms (due to trophic and oviposition) are characterized by necrosis, rusting and bronzing of the fruits. Moreover, WFT spread plant viruses of the genus Tospovirus (TSWV, IYSV).

Recent studies on obligate bacterial symbionts in a few pest thrips species (i.e. *Thrips tabaci*) have shown that relationships insect-associated microbes are an integral part of any proximate or ultimate explanation of insect pest activity. This study is part of a research project that aims to evaluate the effect of the microbiome and endosymbiotic bacteria on biology (feeding, development, fitness) of WFT, in order to suggest new strategies for management of its field populations.

Field sampling of WFT were collected from different crops in two Regions (Campania, Calabria) of South Italy. The molecular characterization of the COI, 28S and ITS2 gene regions was performed in order to evaluate the genetic variability of the WFT populations. Furthermore, through insect small-RNA sequencing and by Denaturing Gradient Gel Electrophoresis (DGGE) technique coupled with 16S-rRNA gene sequencing, the primary symbiotic bacteria of WFT have been identified.

Results on WFT strains variability and identification of some symbiotic bacteria obtain are here reported and discussed.

Further studies will focus on the functional role of these symbionts to develop a tailor-made effective IPM protocols for crops where WFT is the key-pest.

Keywords: WFT, genetic variability, microbiome, symbiotic bacteria

[P2.29]

Bacterial symbionts of Harmonia axyridis: single and multiple infection

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We studied the distribution of bacterial symbionts *Spiroplasma* and *Rickettsia* in seven localities of the native area and six localities of the invasive area of the harlequin ladybird *Harmonia axyridis*. We found that the proportion of beetles infected with *Rickettsia* in native and invasive populations of *H. axyridis* is about 0.03. *Spiroplasma* was found only in native populations of *H. axyridis.* The proportion of infected individuals with *Spiroplasma* in native populations of *H. axyridis* is about 0.08. We discuss the possible influence of *Spiroplasma* and *Rickettsia* in the formation of invasive populations of *H. axyridis*. To identify *Spiroplasma* strains, we analyzed nucleotide polymorphisms of the 16S rRNA gene and the ribosomal internal transcribed spacer (ITS1). We investigated the polymorphism of *Spiroplasma* strains in individual beetles from Kyoto (Japan), Vladivostok, Troitsa Bay, Novosibirsk, and Gorno-Altaisk (Russia). The majority of infected beetles were infected with two or more *Spiroplasma* strains. The abundance of *Spiroplasma* in samples with a single infection is an order of magnitude lower than in samples with multiple infections. Density dependent biological effects of *Spiroplasma* are discussed.

Keywords: symbiont, Harmonia axyridis, Spiroplasma, Rickettsia

[P2.30]

Exploring the developmental origin of butterfly eyespots and the evolution of novel traits

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The origin and evolution of novel traits is an important yet poorly understood phenomenon in evolutionary developmental biology. Novel traits enhance biological diversity and often represent unusual or highly elaborate structures such as beetle horn weaponry, extravagant helmets of treehoppers and eyespots that decorate butterfly wings. A fundamental question regarding the evolution of these traits is whether they evolved gradually in a stepwise fashion or by co-option of a pre-existing gene regulatory network involved in other developmental processes. Butterfly eyespots are stunning morphological novelties and an excellent system to address this question. Eyespots arose once within the Nymphalidae and their origin coincides with the novel expression of a small gene cluster during early eyespot development. These observations raise the question whether eyespots evolved from unique networks wired de-novo gene by gene, or alternatively via a gene network co-option event.

One approach in testing these hypotheses is to identify whether cis-regulatory elements (CRE's) of eyespot-associated genes are pleiotropic or function uniquely in eyespot development. We used an emerging model butterfly, *Bicyclus anynana* to identify genes involved in eyespot development and to elucidate the function of their candidate CRE's. We show using CRISPR-Cas9, that the appendage gene Distal-less (Dll) is required not only for development of legs and antenna but also butterfly eyespots. We then used FAIRE-seq to identify regions of open chromatin found only in wing tissue with eyespots. This approach allowed us identify 12 regions of open chromatin around the Dll gene. Using CRISPR we disrupted one of these CREs and observed a variety of interesting phenotypes including caterpillars with missing thoracic legs as well as butterflies with missing wing, legs and eyespots. In conclusion, our findings support the hypothesis that eyespots evolved via an appendage network co-option event and provide the first functional characterization of a pleiotropic butterfly CRE.

Keywords: Distal-less, Gene network co-option, Novel traits, Butterfly eyespots

[P2.31]

Analysis ofmorphometric parameters for sexdetermination of *Odontobuthus doriae* Thorell 1876 (Arachnida:Scorpionida: Buthidae)), A medically important scorpion from Iran

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Introduction: Mass production of scorpion through development of scorpionarium have been considered in various toxicological and therapeutic departments in recent years. The present study aimed to identify the gender of the important species *Odontobuthus doriae* Thorell 1876.

Methods: This descriptive-analytical study was carried out on the yellow Iranian scorpion *Odontobuthus doriae* which gender has been identified previously through observing delivery in females and spermatophore extrusion in males. In this study, in both gender of this species, the number of pectinal teeth, the pecten length, the fifth mesosomal tergite length and the scorpion body length were compared statistically.

Results: The results of this study showed that the number of pectinal teeth on each side was 31±3 in males and 22±2 in females, length of pecten was 6.5±1.65 mm in females and 8±2 mm in males, the fifth mesosomal tergite length was 10±5 mm in females and 8±2 mm in males, and the scorpion body length was 5.35±2.15 cm in females and 5.35±1.65 cm in males. In males, the number of pectinal teeth and pecten length are greater than that of the females. The fifth mesosomal tergite length is smaller in males than females.

Conclusion: Except the body length, these parameters can be used to differentiate the sex of this scorpion.

Keywords: Scorpion, Odontobuthus doriae, Morphometric Indices, Sexual Dimorphism

[P2.32]

A preliminary study on fauna of medical important solpugid (Chelicerata: Arachnida: Solifugae) in Kashan City, Central Iran

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Introduction: Solpugids are an order of hunter arthropods. They do not have poison glands or sting, but they can hunt animals larger than themselves using their powerful and large chelicerae. Due to their aggressive behavior and scary look, solpugids cause fear and horror in humans. Given the importance of solpugids in terms of causing fear, discomfort and the possibility of infection in case of biting, determining its species was noted in Kashan. This study was a descriptive research.

Methods: Sixty five specimens of solpugids collected from homes and dormitories in Kashan City by hand-collecting method and were transferred to laboratory. All specimens had been collected from public and commuting places .The collected solpugids were preserved in 70% ethanol and were identified using special and valid keys under stereo- microscope in the laboratory.

Results: It was shown that at least 3 species of solpugids from Rhagodidae , Galeodidae and Gylippidae families exist in Kashan. Among 65 solpugids collected , 51% were identified *Galeodes caspius* , 41.5% *Gylippus lamelliger* and 7.5% *Rhagodes melanochaetus*. At least 3 species of solpugids live in 3 families in Kashan that sometimes penetrate the houses and dormitories.

Conclusion: Thus, it is recommended that citizens be trained about the importance of these animals in ecosystem and that they know that these animals are not toxic.

Keywords: Fauna, Solpugid, Iran

[P2.33]

Checklist of Neoneurini (Hymenoptera:Braconidae:Euphorinae) species from China with respectively two new records genera

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Introduction:

The genera *Neoneurus* Haliday,1983 and *kollasmosoma* Achterberg et Argaman ,1993 belongs to the tribe Neoneurini Bengtsson,1918 (Hymenoptera: Braconidae),Which are most likely koinobiont

endoparasitoids of adult ant . In the past Neoneurini were considered to form a separate subfamily, but recent molecular analyses indicate that it is part of Euphorinae Foerster,1862. More than 13 species of the genus *Neoneurus* are known, about half of which occur in the Palearctic. All 4 species of the genus *Kollasmosoma* are Palearctic. In China , the members of the small tribe Neoneurini had only been known to consist of *Elasmosoma* Ruth,1858 and *Sinoeoneurus* He, Chen et van Achterberg,1997. Here, we aim to survey Neoneurinae species diversity of China.

Methods:

The terminology used for measurements and description of morphological characters follows van Achterberg(1988).The photographs were taken with a computer-connected Leica DFC450 digital camera mounted on an Leica M205C stereo microscope.

Results:

1.A key to Chinese genera of Neoneurini was provided.

2.A Check list of Neoneurini species from China was also provided.

2.The genera *Neoneurus* Haliday,1983 and *kollasmosoma* Achterberg et Argaman ,1993 were recorded for the first time from China.

3. The eastern Palaearctic of China *Neonerus aucuts* Thomson,1895 and *Kollasmosoma*

marikovskii (Tobias 1986) is newly record for China.

Discussion:

All of the new records were previously recorded in countries neighbouring China such as Russia(*N. aucuts*) and Kazakhstan(*K. marikovskii*). For the analyses of phylogenetic relationships and cryptic species diversity, more specimens are necessary . The Molecular Taxonomy of the species of Neoneurini is not done.

Keywords: Euphorinae, Kollasmosoma marikovskii, Neonerus aucuts, China

[P2.34]

Desiccation resistance of water beetles from marine supralittoral rockpools

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Desiccation resistance is a key feature of species living in marine supralitoral rockpools subjected to short hydroperiod and strong variation of water temperature and salinity. We analysed the response to drying out of the Hydraenidae aquatic beetles Ochthebius quadricollis and O. subinteger, which cohabiting rockpools in the Iberian Mediterranean coast. We compared their desiccation tolerance (by survival time) and their water loss resistance in adult and larvae of both species. We evaluated the isolated and combined effect of salinity and temperature on the desiccation response variables. After a period of laboratory acclimation to four combined treatments of temperature (20 and 30 °C) and salinity (35 and 90 g/l), individuals were exposed to aerial desiccation (10% RH). In both species, significant effects of temperature in adults survival time were found, being O. quadricollis (23. 69 ± 1.37 h at 20 °C, 6 ± 0.34 h at 30 °C) more tolerant to desiccation than O. subinteger (7.08 ± 0.35 h at 20 °C, 3.69 ± 0.32 h at 30 °C). Both species significantly reduced the survival time when increasing temperature, while salinity and temperature and its interaction had significant effects on water loss rate. At 20 °C, a greater water loss rate was observed in the individuals acclimated to the highest salinity. However, at 30 °C the water loss rate decreased with the increase of salinity in both species, which may indicate acclimation to more extreme conditions of temperature and salinity may confer a higher resistance to desiccation. O. quadricollis supported the greatest loss of water, being more tolerant to desiccation. Similar patterns were found in the larvae, although their survival was noticeably higher than adults, so larvae could be the true resistant state to desiccation, and, consequently, able to recolonise new pools after rewetting.

Keywords: Dessication, Rockpools, Water loss, Survival time

[P2.35]

Identification of blood meal from field collected filarial vector mosquitoes, *Armigeres subalbatus* by multiplex PCR

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Mosquitoes act as vectors of many diseases affecting humans and animals health, which including Zika, malaria, dengue, chikungunya, viral encephalitis, and filariasis. One of the best strategies to control these vector-borne diseases is to control the mosquito vectors. Female mosquitoes require blood source for egg development and the pathogens are released into the vertebrate hosts during female mosquitoes take blood meals. Identification of the types of vertebrate blood sucked by the mosquito is the essential information to develop an effective strategy to control the mosquito populations and also the mosquito borne diseases. Objective of this study was to identify types of mammal blood in *Armigeres subalbatus* mosquito, the principal vector of filarial parasites especially *Dirofilaria* spp.. A total of 210 female *Ar. subalbatus* mosquitoes were collected from the different area of Samed Island, Rayong province, eastern Thailand. Blood meals of the mosquitoes were identified using multiplex PCR technique which is specific to Cytochrome B gene on the mitochondrial. The result of identification types of animal blood from *Ar. subalbatus* from field collected showed that 17.14 % 5.24% 2.38% 0.48% and 10% human, pig, cow, dog and other mammals blood respectively. Avian blood was also detected at 0.95% and 63.81% of the samples were not detected for vertebrate DNA. The benefits of this study are to understand the natural feeding behavior of *Ar. subalbatus* mosquito. Information obtained from the study would be applied to develop the effective control strategies for *Ar. subalbatus* and may provide indirect data suggesting what reservoirs are significant in the mosquito-borne diseases.

Keywords: Armigeres subalbatus, Mosquito, blood meal, Multiplex PCR

[P2.36]

Drosophila seminal fluid peptides revisited

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There is increasing recognition of the importance of non-sperm components of the male ejaculate for reproductive success across animal taxa. Components of the seminal fluid (SF) not only provide a supportive milieu for sperm during transfer and storage in the female, but can also play a key role in reducing sperm competition and ensuring paternity by forming mating plugs and by triggering physiological and behavioral responses in the female, such as a reluctance to re-mating and elevated egg production. In insects, the switching of female behavior on receipt of the ejaculate is generally attributed to signaling peptides made in the male reproductive tract and destined for the SF. Some of these SF peptides are thought to contribute to sexual conflict between males and females and are likely to be subject to selection pressure. In *Drosophila,* these peptides are made by both the paired male accessory glands (MAG) and the ejaculatory duct (ED), and are secreted into the SF along with a wide range of other chemicals including lipids, carbohydrates, nucleic acids and a variety of proteins including enzymes, chaperones, and structural proteins. To achieve this functional diversity, the biosynthetic and secretory pathways of these epithelial cells are likely to be different from classical peptide-producing neuro-endocrine cells and might involve different precursor processing and posttranslational modifications that can make structure predictions of mature peptides from gene sequences more difficult. Quantitative and qualitative variation in posttranslational modification pathways of the MAG and ED have the potential to generate structural variants of SF proteins and peptides which might alter the female post-mating response. We now report on new SF peptide structures and provide insights into the biochemical pathways that lead to extensive posttranslational modification of seminal fluid peptides of *Drosophila.*

Keywords: Drosophila, seminal fluid, peptides, reproduction

[P2.37]

Investigation of biological indicator of soil contamination with heavy metals to assess their ecological condition

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Methods of bioindication and biotesting lead to the appearance in the environment of the detection and determination of biologically significant anthropogenic loads based on reactions to them of living organisms and their communities. Thus, the use of biological methods for environmental assessment involves the isolation of animal or plant species. The method of bioindication using suitable indicator-tore organisms under certain conditions can lead to a qualitative and quantitative assessment (without determining the degree of impact) of the impact of anthropogenic and food impact on the environment.

The use of living organisms as biological indicators. These include the biological activity of the soil (mesofauna, enzymes). Among the parameters of biological activity, the total number of soil mesofauna, the density of rain tissue, the intensity of renal respiration, nitrogen fixation and nitrification should be mentioned.

Bioindicators with chronic anthropogenic stress on the soil, in particular with long-term use, can affect a very weak impact as a result of the accumulating dose, except that they make it unnecessary to use expensive and time-consuming physical and chemical methods for measuring biological parameters.

The purpose of the research is to determine the types of biological indicators depending on the content of various convenient systems.

The data on the mesofauna were obtained, which showed that the common species are insect larvae from the family - *Carabidae, Scarabaeidae, Elateridae, Formicidae*. The dominant species are insect larvae - *Formicidae, Scarabaeidae*. On variants with fertilizers more species are registered. There are more numerous species from the family *Carabida, Scarabaeidae*. It has been established that the quantitative and qualitative composition of the mesofauna is associated with a certain type of soil. A comparative analysis of the mesofauna of light chestnut soils by type of nutrition has been carried out.

Keywords: Insects, Bioindicators, Environmental polution, Soil

[P2.38]

Senwot Wharton, a new genus for the Oriental region with description of two new species from Thailand (Hymenoptera: Braconidae: Alysiinae)

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The subfamily Alysiinae (Hymenoptera, Braconidae) is a large and commonly collected subfamily, which contains over 2,442 described species worldwide (Yu et al. 2016). Monophyly of the Alysiinae is based on the possession of outwardly directed, non-overlapping (= exodont) mandibles combined with endoparasitism of cyclorrhaphous Diptera (Yao et al. 2018). Alysiinae is traditionally divided into two tribes, Alysiini and Dacnusini (Shenefelt 1974; Wharton 1997, 2002). Alysiini (76 genera) contains a greater diversity of lineages than Dacnusini (31 genera), with a wide range of hosts among the Cyclorrhapha (Yu et al. 2016).
Senwot Wharton, 1983, is a small braconid genus with only two previously described species of the subfamily Alysiinae (Sharkey & Wharton 1997; Wharton 1997, 2002; Yao *et al*., 2015a, 2015b; Yu *et al*., 2016). *Senwot* Wharton was described as a monotypic genus with the type species *Senwot africanus* Wharton (1983), from the Democratic Republic of the Congo and Nigeria. A second species, *Senwot fechterorum* Fischer from South Africa was described in Fischer (1997) and is known only from one male specimens.
After intensive collection in Thailand funded by the American National Science Foundation, two new species were found here. We used a combination of morphological data and a phylogenetic analysis of cytochrome *c* oxidase subunit I (COI) barcode (Hebert *et al*., 2003) sequences to delimit our new species.

Senwot Wharton is reported for the first time from Thailand and two new species: *Senwot yinxianggaoae* Yao n. sp. and *Senwot jiyuanyaoi* Yao n. sp. are described and illustrated. These are the first *Senwot* species to be described from outside of the African continent.

The biology of the *Senwot* species is unknown; but all members of the subfamily Alysiinae are koinobiont endoparasitoids of larval cyclorrhaphous Diptera (Wharton, 1984; van Achterberg, 1993).

Keywords: Alysiini, taxonomy, COI, South Asia

[P2.39]

Drought exposure confers resistance to subsequent desiccation in water beetles

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In the context of global change, patterns of rainfall and water availability are expected to change. In Mediterranean areas, drought intensity and duration are predicted to increase. So, understanding desiccation responses should be crucial for aquatic species living in temporal water bodies. For water beetles, whose adults disperse by flying from drying sites to more favourable wet habitats, control of water loss through the cuticle is the main mechanism of desiccation resistance. We explored acclimation responses to desiccation stress in adults of two species of saline water beetles *E. jesusarribasi* (Hydrophilidae) and *N. baeticus* (Dytiscidae). Individuals were exposed to two different aerial desiccation treatments: rapid desiccation at 10% HR for 1.5h and slow desiccation at 40% RH for 6h, plus a control treatment at 100% HR. After a recovery period resubmerged in water from collection sites for 24h, specimens were exposed to a subsequent desiccation treatment (40% HR for 12h in *E. jesusarribasi*, and 6h in *N. baeticus*). Survival and water loss were measured after each phase and at different time-points along the second desiccation treatment. The rapid desiccation produced a higher initial water loss rate than the slow desiccation in both species. Individuals of both species from the slow desiccation treatment showed lower water loss rates under the subsequent desiccation than the control group, but such acclimation response was not observed after the rapid desiccation. In *N. baeticus* survival during the second desiccation exposure was also significantly higher in individual previously desiccated. These acclimatation responses could be associated with changes in cuticular hydrocarbons (CHC) composition causing a decrease of cuticle permeability to controling water loss.

Keywords: desiccation, water beetles, acclimatation

[P2.40]

The killer yeast *wickerhamomyces anomalus* is a potential new tool for the symbiotic control of malaria

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Introduction

Wickerhamomyces anomalus is a yeast associated with different insects including disease vector mosquitoes and sandflies (Ricci *et al*. 2011; Martin *et al*. 2015), where it is proposed to be involved in symbiotic relationships with hosts (Cappelli *et al*. 2014; Martin *et al*. 2018). Different symbiotic strains of *W. anomalus* display a killer phenotype mediated by protein toxins with broad-spectrum antimicrobial activities. In particular, a killer toxin (KT) purified from a *W. anomalus* strain (*Wa*F17.12), isolated from the malaria vector mosquito *Anopheles stephensi*, has shown anti-plasmodial activities against early sporogonic stages of the murine malaria parasite *Plasmodium berghei*.

Methods

*Wa*F17.12 was cultured in selective conditions to stimulate KT production. Supernatants were separated by chromatography and analysed using an antibody specific for yeast KTs. Food preparation containing activated *Wa*F17.12 was provided to *An. stephensi*. The effect of *Wa*F17.12-KT was tested using *P. berghei* transgenic strains that express Green Fluorescent Protein. Twenty-four hours after the infected blood meal, mosquito guts were analysed through fluorescence microscopy to detect early sporogonic stages.

Results

*Wa*F17.12 cultures, properly stimulated to induce the KT expression, affect *P. berghei* early sporogonic stages in the mosquito midgut lumen, causing parasite membrane damage and death. A mosquito dietary supplementation with activated *Wa*F17.12 cells, strongly interfere with the ookinete development, significantly reducing the parasite number.

Discussion

Innovative biotechnologies propose novel tools for the prevention of insect-borne diseases as the Symbiotic Control (SC), which implies the use of microbial symbionts living in insects to block pathogens transmission. Symbionts can be used as a Trojan horse to drive antagonist molecules in the vector's gut, interfering with pathogens proliferation. *Wa*F17.12 might be a good candidate for SC and its strong anti-plasmodial activity, observed in our study, outlines further investigations in wild vectors, that could lead to innovative, safe and cost-competitive tools against malaria.

Keywords: malaria, mosquito, yeast, symbiotic-control

[P2.41]

The physiology and ecology of thermal tolerance limits in insects

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Insects represents the majority of all animal species and play critical roles in most ecosystems and in interactions with human society. Environmental temperature is arguably one of the most important factors dictating the distribution of insects. Considering the projected changes in global climate, it is important to understand the ecological and physiological limitations that determine distribution limits of insects.

In this presentation, I will firstly discuss how measures of thermal tolerance and thermal performance can be used to model insect distribution. I will show how the use of acute thermal limits at temperature extremes can be used to model current distributions of *Drosophila* species while chronic measures of population growth potential are much harder to use for modelling purposes. Having established the importance of acute thermal tolerance I will highlight how specific physiological systems are particularly important in setting the upper and lower thermal limits, respectively. In this discussion, I will compare physiological capacities of *Drosophila* species with different thermal tolerance to heat and cold. With regard to cold tolerance, I will discuss how the physiological capacity of the osmoregulatory system is critically important for the maintenance of homeostasis at low temperature. Some species are able to continue osmoregulation at low temperature when other species fail leading to a loss of ion balance that causes neuromuscular failure and mortality. With regard to heat tolerance, I will give examples of how adaptations of central nervous function are tied to heat tolerance in drosophilids and present new data that compares heat mortality, protein inactivation and loss of CNS function in tolerant and sensitive species. With this presentation I hope to inspire a discussion on how we can best asses insect thermal tolerance and also highlight how particular physiological systems may be more rewarding to study in this context.

Keywords: Temperature, Physiology, Climate change

[P3.02]

Plant Cell Wall Degrading Enzymes Genes from The Boll Weevil, *Anthonomus grandis* (Coleoptera: Curculionidae)

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During evolution, plant and pests co-evolve together developing mechanisms to survive to each other. Some herbivorous insects have acquired, by horizontal gene transfer (HGT), enzymes able to degrade plant biomass. Cotton is one of the most important commodity in the world and it is severely attacked by the coleopteran *Anthonomus grandis* in Americas. This pest causes great damage to cotton flower bud and boll reducing the quality of cotton fiber. In this work we have focused especially in identifying plant cell wall degrading-enzymes (PCWDE), especially cellulases and pectinases, and how these genes are expressed along different *A. grandis* tissues. We have searched for PCWDE sequences in *A. grandis* midgut transcriptome previously sequenced using next-generation sequencing technology. We have found these genes using tBlastN tool with PCWDE protein sequences from other insects as queries. We have identified contigs belonging mainly to glycosyl hydrolase (GH), carbohydrate esterase (CE) and pectate lyase (PL) enzyme families. Specifically, we have found 11 contigs homologous to GH28, five to GH45, four to GH48, two contigs homologous to CE, and three to PL4. We have analyzed the phylogenetic relationship of these genes to show their close relation with microbe genes. Furthermore, we have validated these genes expression by RT-qPCR. Most of genes were expressed majorly in anterior midgut portion. Finally, we have studied in more detail how carbohydrate esterases are expressed at *A. grandis* different life stages and validated if these genes were essential for *A. grandis* development using RNA interference (RNAi). We have proved that, like in other curculionids, *A. grandis* has a whole PCWDE arsenal probably used to degrade cellulose and pectin present in cotton bolls and flower buds. These findings could be used to develop new tools to aid in efficient control of this pest, and possibly help production of ethanol from plant biomass.

Keywords: Herbivory, Cellulase, Pectinase, Insect control

[P3.03]

Could the exposure to a sublethal dose of imidacloprid induce cellular and physiological changes leading to cockroaches adapted to their environment?

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Introduction: To ensure that enough food is available to meet the needs of a growing population, insecticides have been used. However, widespread insecticide treatments have led to resistant insect emergence. Studies were focused mainly on the mechanisms involved in resistance. We know now that exposure to sublethal doses of insecticide can induce physiological changes favouring the development of adapted insects. Indeed, as insecticides degrade over time or can volatilize with wind until their concentration becomes sublethal, insects may be exposed to different doses. Therefore, the aim of our study is to identify cellular and molecular changes induced by subchronic exposure to a sublethal dose of insecticide and to explore if these modifications are maintained over time to promote the development of resistant insects.

Methods: The *Periplaneta americana* cockroaches were exposed to a sublethal dose of insecticide (imidacloprid) during 30 days following by a resting period of 30 days without insecticide. Effects of this treatment were assessed at 30 and 60 days. Three doses (referred as LD_{10}, LD_{50}, LD_{90}) were used to determine mortality rate of non-treated and treated cockroaches at 24h, 48h and 72h.

Results: We demonstrated that the subchronic exposure to a sublethal dose of imidacloprid during 30 days reduces sensitivity to imidacloprid of treated cockroaches compared to control cockroaches. This decrease of sensitivity to insecticide was also observed on treated cockroaches after the 30-day resting period.

Discussion: Exposure to sublethal dose of insecticide induces physiological modifications lead to decrease imidacloprid efficacy on treated cockroaches. Further studies on cellular and molecular changes, such as increased activity of detoxification enzymes or modification of the insecticide targets, that may be involved in adaptive mechanisms will be considered to develop innovative strategies to circumvent insecticide resistance and to improve pest control.

Keywords: Insecticide, Sublethal dose, Adaptation, Cockroaches

[P3.04]

Candidatus Liberibacter asiaticus gene expression in gut of nymphs and adults of *Diaphorina citri*.

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Huanglongbing (HLB) is the major threat to the citriculture worldwide. The fast and efficient dissemination of the HLB associated bacteria, *Candidatus* Liberibacter spp., by its psyllid vector *Diaphorina citri* and the absence of genetic resistance in citrus, are major factors accounting for disease severity. Our goal was to identify the expression of genes of *Candidatus* Liberibacter asiaticus in the gut of psyllids after different periods of acquisition in HLB symptomatic plants. Changes in the pattern of gene expression of *Ca.* L. asiaticus were evaluated separately by comparing the expression in nymphs and adults of infected *D. citri*, and the expression of *Ca*. L. asiaticus genes at three different periods of plant feeding by adults (1-2 d, 3-4 d, and 5-6 d). Insects from each feeding period were collect and subjected to RNA extraction, RNA samples were enriched in mRNA, and sequenced in an HiSeq2500 Illumina platform using a paired-end approach (2 x 100bp). Differential expression of *Ca*. L. asiaticus genes was performed using the tools implemented in the software CLC Genomics Workbench 12.0, by counting the number of reads per kilobase of the target sequence by million of obtained reads (TPM – transcripts per kilobase million) against the reference genome of *Ca*. L. asiaticus (NC_012985, psyllid psy62). The highest number (31 transcripts) of genes differentially expressed was verified in adults at the early (1-2 d) and late (5-6 d) of feeding. Comparisons between L. asiaticus expression in nymphs and adults identified 13 differentially-expressed genes. Our data provides information that could help with the understanding of the process of acquisition and infection of *D.citri* by *Ca*.L. asiaticus.

Keywords: Huanglongbing, Asian citrus psyllids, infection establishment

[P3.05]

Elucidation of molecular expression associated with abnormal development and sterility caused by electron beam irradiation in *Helicoverpa armigera*

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Helicoverpa armigera Hübner (Lepidoptera: Noctuidae) is an economically important pest, which harms various kinds of important agricultural crops, such as cotton and tomato. Electron beam radiation on *H. armigera* was performed to assess developmental inhibition and to identify a potential quarantine treatment dose of the radiation. Electron beam radiation treatment at different doses of 50, 100, 150, 200, and 300 Gy was carried out with egg, larvae, pupae and adults of *H. armigera* . Electron beam radiation induced developmental inhibition of all stages of *H. armigera*. When larvae were treated with the electron beam, morphological deformities appeared in the pupae, and abnormal wing disc (AWD) expression significantly decreased. Ovarian development was completely inhibited in emerged adults that had undergone 200 Gy electron beam irradiation as pupae. Quantitative real-time PCR (qRT-PCR) assays showed significant down regulation of the Vg and VgR genes due to electron beam irradiation; whereas the synthesis level of Vg protein did not decrease with time in eggs unlike in non-irradiated (control) *H. armigera*, exhibiting irradiation induced impairment of Vg functioning. These findings of radiation-induced abnormal development and sterility in *H. armigera* together with the correlated changes at the molecular level may facilitate the development of a phytosanitary strategy against this quarantine pest using electron beam irradiation.

Keywords: Electron beam, Helicoverpa armigera, sterilization, abnormal development

[P3.06]

The development of nanocarriers to improve RNAi efficacy in insect pests

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The development of nanocarriers to improve RNAi efficacy in insect pests

INTRODUCTION: Although several insects, notably coleopteran species, have shown a high sensitivity to dietary uptake of naked double-stranded RNA (dsRNA), many other important pest insects are more refractory to RNA interference (RNAi). In this research, different novel dsRNA delivery strategies were developed, including novel polymer- and protein-carriers with the aim to improve RNAi efficacy in different insect species, such as the lepidopteran *Spodoptera exigua* and the hemipteran *Euschistus heros*.

MATERIALS AND METHODS: First, the ability of the carriers to protect dsRNA against nucleolytic degradation in midgut juice or saliva was investigated in vitro. Next, in vivo feeding bioassays were performed to confirm their ability to increase oral RNAi efficacy. Finally, CF203 cell culture confocal microscopy experiments were carried out to investigate the rate and mechanisms of cellular uptake of dsRNA.

RESULTS: Our data show that some of these nanocarriers protect dsRNA in harsh nucleolytic environments and significantly increase RNAi efficacy at transcript level (up to 10-fold) and in terms of mortality (up to 3-fold). Unexpectedly, the confocal microscopy experiments also demonstrated that some carriers can directly increase the rate of cellular uptake in insect midgut cells. Polymer- or lectin-complexed dsRNA for example required less than 10 minutes to be internalized in the cells in these assays, while for naked dsRNA the first uptake was visible after 30 minutes incubation.

DISCUSSION: Here, we show that different nanocarriers can increase the efficacy of oral RNAi in selected insect species, leading to potential new improvements for RNAi-based pesticides. Furthermore, our research also leads to novel insights in the cellular uptake mechanisms of dsRNA in insect cells, showing that both clathrin-mediated endocytosis and caveolae-mediated endocytosis appear to be involved at the same time.

Keywords: RNA interference, pest control, nanocarriers, dsRNA cellular uptake

[P3.07]

The genetic basis of toxin resistance in *Drosophila sechellia*

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Drosophila sechellia is a species of fruit fly endemic to the Seychelles islands. Unlike its generalist sister species, *D. sechellia* has evolved to be a specialist on the host plant *Morinda citrifolia*. This specialization is interesting because the plant's fruit contains secondary defense compounds, primarily octanoic acid (OA), that are lethal to most other Drosophilids. Although ecological and behavioral adaptations to this toxic fruit are known, the genetic basis for evolutionary changes in OA resistance are not. Prior work showed that 5 genomic regions contribute to this trait with a genomic region on chromosome 3R containing 18 genes having the greatest contribution to differences in OA resistance between resistant species *D. sechellia* and sensitive sister species *D. simulans*. Using protein inactivation, we ruled out the contribution of the well known cytochrome P450 and glutathione S-transferase gene families to this trait change and discovered that one or more genes in the esterase gene family does contribute evolved resistance in *D. sechellia.* To further interrogate the fine-mapped QTL region on chromosome 3R, we used a combination of RNA-seq and an RNAi gene expression knockdown screen and identified three neighboring genes in the Osiris family, *Osiris 6* (*Osi6*), *Osi7*, and *Osi8*, that lead to decreased OA resistance when ubiquitously knocked-down in adults. Further dissection of the role these genes play in OA resistance found that tissue-specific environmental plasticity of *Osi6* gene expression, evolved constitutive changes in gene expression of *Osi7*, and protein coding changes in *Osi8* may all contribute to OA resistance in *D. sechellia*. Osiris represents a new class of genes implicated in evolved insect resistance to chemicals and ongoing studies in the lab are investigating the biochemical, cellular and genetic mechanisms involved in evolution of toxin resistance through this newly described gene family.

Keywords: Host specialization, Toxin resistance, Evolutionary genetics

[P3.08]

Antichymotrypsins: A potential saviour of *Helicoverpa armigera* from plant protease inhibitors

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Introduction: *Helicoverpa armigera* (Lepidoptera: Noctuidae) is a major pest worldwide. Among various control strategies, plant protease inhibitors (PIs) are one of the promising phytochemicals against lepidopteran insects. However, *H. armigera* is known to adapt to PIs by differentially regulating their digestive proteases but the mechanisms involved in regulation are largely unknown in insects. This knowledge in lepidopteron insects is the key to increase potency of plant PIs and also for alternative pest management strategies. Previous studies suggest the potential role of endogenous PIs of *H. armigera* in protease regulation. However, this study needs to be validated. Here, we examined an antichymotrypsin protein (serpin superfamily) which might be involved in post translational regulation of proteases.

Methods: First instar larvae were fed on artificial diet (AD) or recombinant CanPI (pin-II type PI from *Capsicum annuum*) incorporated AD. Hemolymph was collected from fourth instar larvae and shotgun proteomics was performed using SWATH. Antichymotrypsin gene was amplified from CanPI fed insects, cloned and expressed in BL21(DE3) cells. Activity assays were performed using synthetic substrate and gel X-ray film contact (GXFC) method.

Results: Proteomics study indicates differential expression of antichymotrypsin upon CanPI ingestion. This was confirmed by activity assays using GXFC method followed by protein identification using mass spectrometer. Recombinant protein was more potent at pH 10 against *H. armigera* gut proteases (IC_{50}=11nM) than bovine chymotrypsin at pH 6.4 (IC_{50}=18nM, Fig. 1). Genome investigation revealed the presence of 18 isoforms, varying in exon 9 containing the reactive centre loop suggesting alternative splicing (Fig. 2). Further experiments are planned to understand specificity of each isoform and their role in protease regulation.

Fig 1

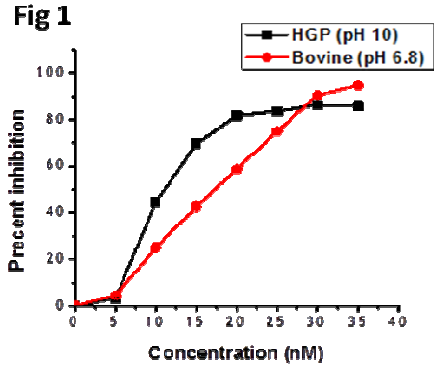
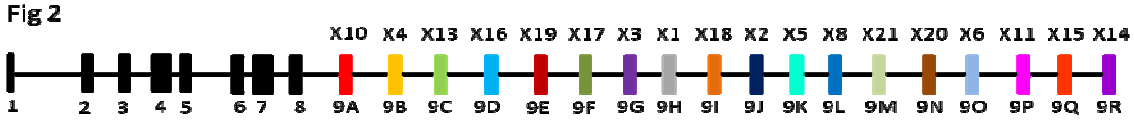


Fig 2



Discussion: In accordance with previous hypothesis of protease regulation by peptide hormones, antichymotrypsin might play a vital role in hijacking the feedback regulation by inhibition the regulatory protease in hemolymph.

Keywords: Protease regulation, Helicoverpa armigera, Proteomics, Protease inhibitors

[P3.09]

Dissecting insecticide resistance via genetic manipulation and genome modification in Drosophila

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The protection of agricultural production, as well as the prevention of vector borne diseases, largely relies on the control of insect populations with the use of insecticides. However, insects display an intriguing ability to develop resistance. Our research aims at the investigation of the mechanisms inducing resistant phenotypes using several experimental approaches; among these, genetic transformation and genome modification in model species like *Drosophila* has played a pivotal role in the understanding of the role of individual alleles and the determination of specific insecticides' mode of action. Certain examples include the investigation of the contribution of specific mutations in target-site resistance phenotypes (in genes like chitin synthase, ryanodine receptor, ACCase and voltage-gated sodium channels) using CRISPR/Cas9 genome modification. Furthermore, the heterologous GAL4/UAS overexpression of candidate detoxification genes, facilitated by *Drosophila* genetic transformation, is invaluable for the validation of particular genes implied in resistance. Among recent examples, the contribution of individual cytochrome P450s in insecticide detoxification in major agricultural pests was investigated.

The versatility of these tools is demonstrated by the ability to devise *ad hoc* strategies in order to test hypotheses where the emergence of resistance may involve several different mechanisms. Such cases may be dissected in-depth with the combined use of genome modification, conditional expression and/or knock-down (via RNAi) in the same genetic context, aiming to reconstruct resistance pathways in a susceptible genetic background, taking advantage of the unique available genetic toolkit and standard *Drosophila* genetics.

Despite certain limitations, this approach greatly enhances our ability to investigate insecticide resistance in a fashion complementary to standard pipelines investigating resistance in the field and classical forward genetics. Engineered "super-resistant" *Drosophila* lines, bearing several resistance genes and/or mutations simultaneously and showing striking resistance phenotype against classical insecticides, can eventually be used to test the anti-resistance potential of novel candidate compounds, synergists etc.

Keywords: Insecticide Resistance, CRISPR/Cas9, cytochrome P450s, Drosophila model

[P3.10]

Investigation of abamectin resistance following expression of Tetranychus urticae CYP392A16 in Drosophila

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Agricultural production protection largely relies on the control of pest populations with the use of insecticides. However, pests display an intriguing ability to develop resistance through several mechanisms that may include target-site alterations and/or overexpression of detoxification genes. Abamectin resistance in the two spotted spider mite *Tetranychus urticae* has been associated with target-site mutations in the Glutamate-gated chloride channel (GluCl) as well as with overexpression of certain cytochrome P450s, like CYP392A16 which has been shown to metabolize abamectin *in vitro*.

In order to validate the role of CYP392A16 *in vivo* we performed heterologous GAL4/UAS overexpression of the protein (together with the relevant associated TuCPR) in transgenic *Drosophila*, with the use of Gal4 drivers driving expression in detox-related tissues. We performed toxicity bioassays to investigate the ability of CYP392A16 to confer abamectin resistance *in vivo*.

Furthermore, we are in the process of generating relevant *Drosophila* GluCl mutants aiming to validate confounding abamectin resistance mechanisms in a susceptible genetic background, taking advantage to this end of the unique available genetic toolkit and standard *Drosophila* genetics.

Despite certain limitations, this approach may significantly enhance our ability to investigate complex insecticide resistance phenotypes by engineering *Drosophila* lines bearing several resistance genes and/or mutations simultaneously.

Acknowledgement: This research is co-financed by Greece and the European Union (European Social Fund- ESF) through the Operational Programme “Human Resources Development, Education and Lifelong Learning 2014-2020” in the context of the project “Validation of the contribution of individual mechanisms to the resistance of agricultural pests to insecticides, as well as the role of diagnostics in the control programme management” (MIS 5004351).

Keywords: Insecticide Resistance, CYP392A16, Tetranychus urticae, Drosophila model

[P3.11]

Double-stranded RNA complexation with well-defined block copolymers: enhancing stability and uptake for use in pest control

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Without significant advances in crop-protection, it is likely that food production will not be able to sustain an ever-increasing world population. Unfortunately, environmental concerns and the increasing frequency of pesticide resistance means our current arsenal of insecticides is unlikely to be sufficient. As a result, it is important to consider alternatives to existing chemical pesticides. Biopesticides derived from natural sources with new modes of action, such as peptide/protein toxins, hormone analogues and double-stranded RNA (dsRNA) mediated gene silencing by RNA interference (RNAi) have been proposed as alternatives. RNAi has a number of advantages over conventional chemical insecticides, such as selectivity for a target pest, however, efficacy can vary from species to species, and its wide-scale use is currently limited by its rapid degradation upon ingestion and suffers from poor uptake from the gut.

By complexing dsRNA to specific block copolymers containing a complexing and stabilising block, we propose to increase the protection of the dsRNA to degradation from extracellular nucleases and increase RNAi efficiency. Specifically, we have produced well-defined diblock copolymers comprising poly(2-hydroxypropyl methacrylamide) –b-poly((dimethylamino)ethyl methacrylate) (PHPMA-b-PDMAEMA) which are subsequently quaternised. We have investigated their complexation with dsRNA targeting the gut vATPase. Phenotypic observations of the increased stability and penetration was undertaken for both complexed and naked dsRNA targeting the expression of the *Drosophila suzukii* gene. It was found that complexation protected the dsRNA from gut nucleases and strongly enhanced the lethality of the dsRNA, resulting in 75% mortality when incorporated into the larval diet. Feeding of the complex to *Drosophila melanogaster* had no adverse effects on larval and pupal development of this related fruit fly, demonstrating the advantageous selectivity of this complexed dsRNA. Our approach has the potential to greatly improve the efficacy of dsRNA as an oral bioinsecticide.

Keywords: RNA interference, Drosophila suzukii, insecticide, complexing polymers

[P3.12]

Monoterpenes can modulate type 1 tyramine receptor in *Drosophila suzukii* (*DsTAR1*): molecular and pharmacological aspects of new possible biopesticides.

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Monoterpenes, the major components of plant essential oils, have interesting insecticidal properties against various insect species. They act their toxic effects through multiple mode of action, such as AchE inhibition, blockage of $GABA_A$ receptors and interaction with tyramine and octopamine receptors. Type 1 tyramine receptors (TAR1) are G-protein coupled receptors implicated in several physiological processes in insects, such as locomotion, olfactory learning and metabolism.

Recently we have characterized the first type 1 tyramine receptor from the pest *Drosophila suzukii* (*DsTAR1*) with the aim to study a possible insecticidal target for biopesticide, such as monoterpenes.

In this work, we have used HEK293 cell line stably expressed *DsTAR1* as a system for testing some monoterpenes (thymol, carvacrol, α-terpineol) as agonists or antagonists / modulators of this receptor.

All three terpenoids showed no evidence as agonists in both Dynamic Mass Redistribution (DMR) assay and Calcium Mobilization assay. Conversely in DMR assay, they increased the potency of the endogenous ligand tyramine (TA), when $HEK293_{DsTAR1}$ were pre-incubated with 10 μM and 1 μM of monoterpenes. In particular, 1 μM of α-terpineol was able to increase the potency of TA five-fold compared to the control ($HEK293_{DsTAR1}$ pre-incubated with buffer).

Expression analysis on adults *D. suzukii* exposed for 24 h, 72 h or 120 h at sublethal concentration of monoterpenes (1 mg/l) showed a down-regulation of *DsTAR1*. Significant differences were observed for α-terpineol at 24 h, thymol at 72 h and for carvacrol at 120 h.

These data allowed us to develop the hypothesis according to which the down-regulation of TAR1, observed in *D. suzukii*, represents a compensation mechanism activated in response to the increased potency of the endogenous ligand TA, caused by monoterpenes. This hypothesis, if confirmed, we may expect to open new scenarios in the fight against *Drosophila suzukii*.

Keywords: Drosophila suzukii, Tyramine receptor, Monoterpenes, Dynamic Mass Redistribution

[P3.13]

Type 1 tyramine receptor (TAR1) from the brown marmorated stink bug *Halyomorpha halys*: characterization of a possible new target for biopesticides.

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The brown marmorated stink bug, *Halyomorpha halys* (Heteroptera: Pentatomidae) is native to China, Korea and Japan and was recently introduced into Europe and the United States. This invasive insect has quickly become one of the major agriculture pests, being able to attack over 100 different plant species. Furthermore, its control is challenging by either chemical methods as well as containment strategies. Unfortunately, *H. halys* studies are still limited and, for this reason, its physiology and behavior are less known.

A possible target for the development of new control methods against insects is represented by the tyramine/octopamine receptors. Octopamine (OA) and tyramine (TA) amines are present in traces in vertebrates, while in invertebrates they act as substitutes for adrenaline and noradrenaline. Indeed, these amines regulate numerous physiological processes in insects. They exert their effects by binding to specific receptor proteins that belong to the superfamily of G-protein coupled receptors (GPCRs).

Here we report the cloning and the preliminary characterization (at the molecular and pharmacological levels) of the gene coding for *Halyomorpha halys* type 1 tyramine receptor (*HhTAR1*). The cDNA is 1.35kb long and codes for a 448 amino acid polypeptide featuring seven transmembrane domains, as expected for a GPCR.

HhTAR1 deduced sequence was compared to the amino acid sequence of octopamine/tyramine receptors from other insects and the various receptor sequences were characterized by phylogenetic analysis.

A preliminary genetic screening will be presented on bugs collected in the different orchards to evaluate the population genetic profile focusing on the *HhTAR1* gene and other genes involved in detoxifying system.

A stable HEK293 cell line stably expressing *HhTAR1* was tested for responsiveness to the endogenous agonist tyramine (TA) and to the antagonist yohimbine by two different pharmacological approaches, the Intracellular Calcium Mobilization assay and the Dynamic Mass Redistribution (DMR) assay.

Furthermore, the expression level of the receptor was studied by qRT-PCR analysis in different organs of adult *Halyomorpha halys* male and female (antennae, brain, midgut, malpighian tubules, females or males reproductive organs). Concerning the antennae, a preliminary investigation by scanning electron microscopy was also conducted to study the distribution and morphology of sensilla in both sexes.

HhTAR1 represents the first GPCR characterized in *Halyomorpha halys* and its study will help understand the role of TA in the physiology and behaviour of this dangerous pest and to possible development of novel insecticides.

Keywords: Brown marmorated stink bug, Halyomorpha halys, Type 1 tyramine receptors, Biopesticides

[P3.14]

Evaluation of Beauveria bassiana, Metarhizium anisopliae, and Bacillus thuringiensis for the Management of Helicoverpa armigera (Hubner) (Lepidoptera: Noctuidae)

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The pod borer, *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae), is a common and destructive insect pest of chickpeas that has proven difficult to control using conventional insect control methods. Experiments were performed to evaluate the pathogenicity and virulence of 14 isolates of *Beauveria bassiana* and *Metarhizium anisopliae* and a commercialized wettable product of *Bacillus thuringiensis* against larvae of *H. armigera*. Three fungal isolates of *B. bassiana*, *APPRC-9604, APPRC-T5* and *DLCO-EA-56,* were most effective in causing high larval mortality to third instar *H. armigera* larvae when a single concentration assay was conducted. The *B. bassiana APPRC-9604* fungal isolate had the highest virulence against the third larval instar of *H. armigera*. Field trials indicated that *B. bassiana* is effective under field conditions, reducing larval infestations, decreasing pod damage and subsequently increasing chickpea yield. Concentrations of wettable of *Bt.* caused higher mortality to second instar than to third instar *H. armigera* larvae. Larvae feeding on *Bt.*-treated chickpea leaves required additional days for pupation and adult emergence and had a shortened adult life span compared to those of untreated larvae. From the present laboratory and field study, we concluded that three fungal isolates of *B. bassiana,* particularly the *APPRC-9604* isolate, and commercialized *Bt.* showed high potential as biological control agents of *H. armigera* larvae. Thus, further research should focus on the efficacies of commercialized *Bt.* under field conditions.

Keywords: Biological control, Chickpea, Entomopathogens, Isolates

[P3.15]

Enhancing the efficiency of SIT by feeding dsRNA of sexual fate genes in *Anastrepha ludens* (Loew)

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New technologies are needed to improve fruit fly pest control methods. The Sterile Insect Technique is widely accepted to reduce insect densities. A technical limitation is that females must be sorted before males are released, to compete with wild females. The Mexican fruit fly, *Anastrepha ludens* (Loew), a is a major pest in America. A SIT program currently separates female and male pupae through sex-specific color sorting. In Tephritids, silencing of sex determining genes produces pseudomales, ovipositor deformations or male-only progeny. We propose to incorporate RNA interference in the SIT process to improve overall efficiency. In this study, bacterial-produced dsRNA of the sex determination genes *transformer* (*Altra, Cctra*) and *transformer-2* (*Cctra-2*) were delivered orally in water to *A. ludens* Tap 7 females during their early sexual maturation period, 11 days post-emergence. Feeding of dsRNA producing bacteria significantly affects the pupae-adult molt, but does not affect total egg production. We observed a 67-63% significant decrease in numbers of F1 adults after feeding target and control dsRNA producing bacteria compared to flies given water or bacteria without dsRNA production. We found 0.27-0.62% ovipositor deformations amongst F1 females exposed to *tra* or *tra-2* dsRNA treatments. Future studies will optimize feeding conditions and synchronization with sexual maturation and during mating. Our results suggest that targeting the expression of the transformer genes could become a cross-species strategy to reduce the risk of releasing fertile females.

Figure 1. Experimental procedure

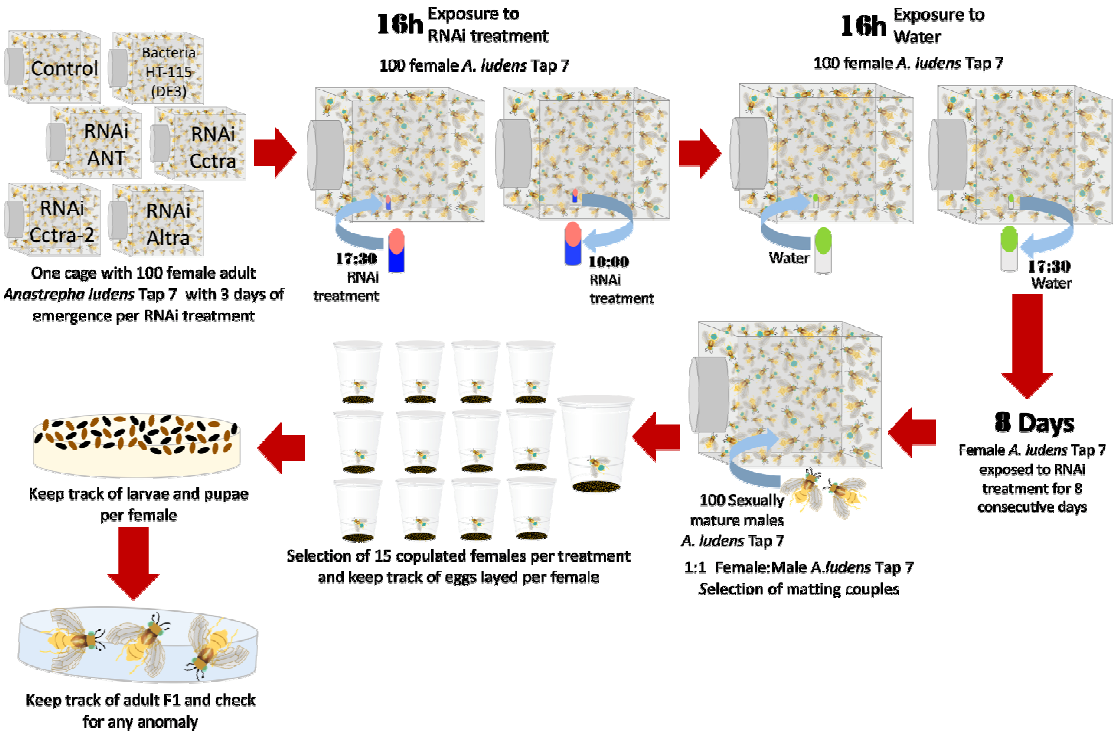
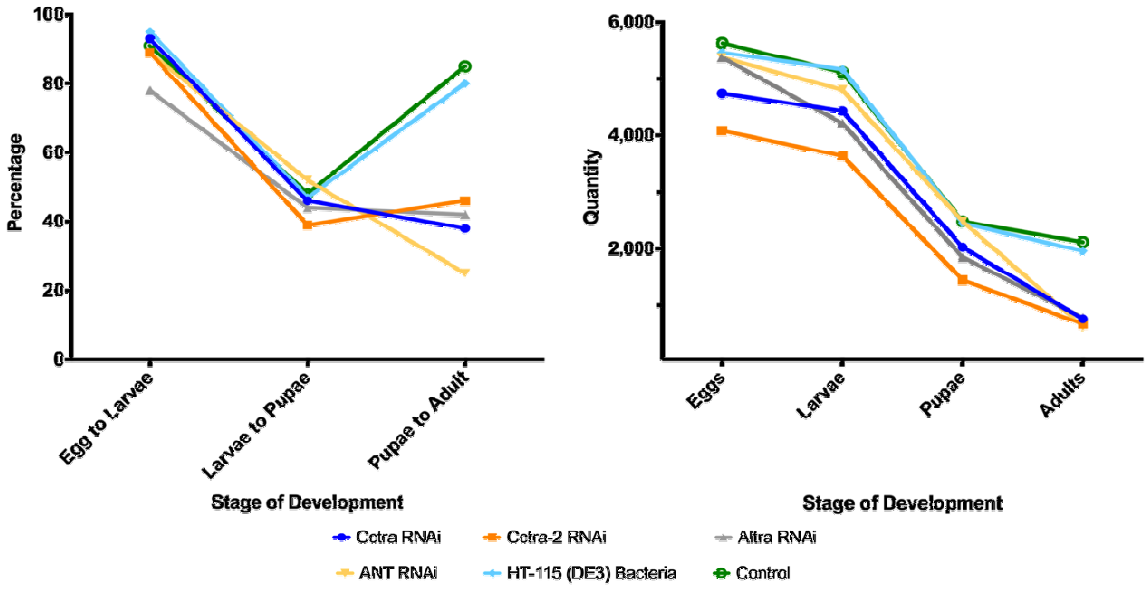


Figure 2. Oral delivery of dsRNA affects viability of *Anastrepha ludens* pupae.

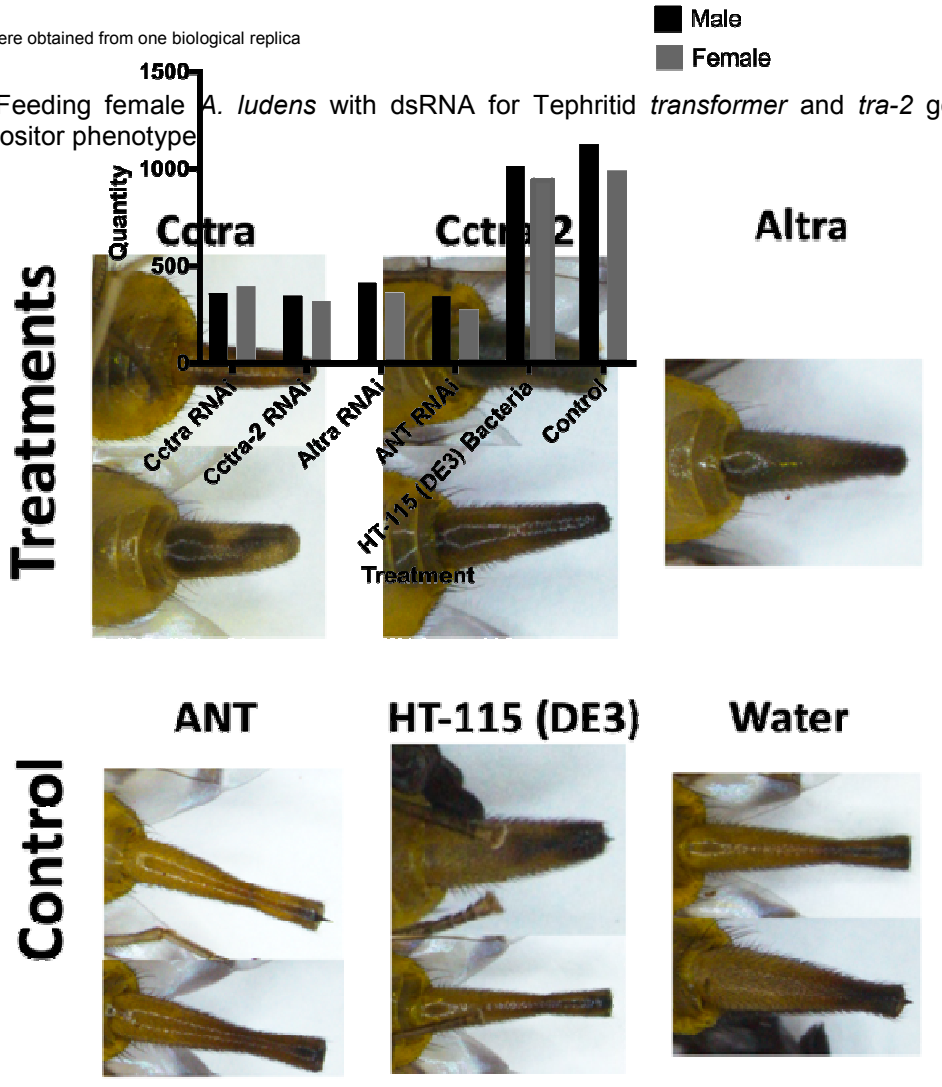


* These results were obtained from one biological replica

Figure 4. Oral delivery of dsRNA affects number of F1 female and male adults

* These results were obtained from one biological replica

Figure 5. Feeding female *A. ludens* with dsRNA for Tephritid *transformer* and *tra-2* genes affects ovipositor phenotype



Keywords: dsRNA, transformer, SIT, Anastrepha ludens

[P3.16]

Comparative efficacy of the entomopathogenic fungus, Beauveria bassiana (Bals.) Vuill. and Metarhizium anisopliae (Metchnikoff) Sorokin on larval mortality, enzyme inhibition of Spodoptera litura Fab. and their non-target activity against Eudrilus eugeniae Kinb

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Biopesticides are necessary for the regulation of endemic and invasive pests impacting India, including those that are emerging as a result of climate change and farming intensification. Entomopathogenic fungi are feasible both as systems for the control of insect pests in agriculture with a growing market and as an important model for studies of host-pathogen interaction. At the same time, important progress has occurred in the understanding of the molecular aspects of the pathogenesis and in the development of tools to validate putative virulence factors by the construction of over-expressing or knock-out strains. In the present study is focused on the comparative efficacy of entomopathogenic fungi *Beauveria bassiana* (Bals.) Vuill. and *M. anisopliae* against *S. litura* (Fab.) through the assessment of larvicidal and antioxidant enzyme inhibition and the non-target screening of entomopathogenic fungi against the beneficial earthworm, *E. eugeniae*, in comparison to commercial pesticides. The entomopathogenic fungus exposure resulted in the modification of the levels of detoxification enzymes as well as significant increases in Superoxide Dismutase (SOD) and Catalase (CAT) activity after exposure of entomopathogenic fungus. Bioassay results showed that *B. bassiana* and *M. anisopliae* affect third and fourth instar larvae of *S. litura*. The artificial soil assay of non-target beneficial organism, the soil indicator earthworm E. eugeniae, with fugal culture of *B. bassiana* (5×10^8 conidia/ml/kg) and *M. anisopliae* (5×10^8 conidia/ml/kg) showed no toxicity compared to Monocrotophos at the dosage of 10 ppm/kg. Current results suggest that mycotoxins of *M. anisopliae* and *B. bassiana* are able to significantly reduce the lepidopteran pests while having only low toxicity to other beneficial species.

Keywords: Entomopathogenic fungi, insecticide, Detoxification, earthworm

[P3.17]

Quantifying the amount of plant subcellular fractions ingested by the four sucking pest species following time course.

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Although thrips and mites are known as cell-feeding sucking pests, little information has been available yet on how much of subcellular fractions they can ingest. In this study, therefore, the ingested amount and time course of plant subcellular fractions in four sucking pest species (*Frankliniella occidentalis*, *Frankliniella intonsa*, *Tetranychus urticae* as cell feeders and *Nilaparvata lugens* as a sap feeder reference) were determined. Adults of the four species were starved for 24 h and then fed with appropriate host plants (leaf of kidney bean and rice) for 48 h. The leaf-fed insects were collected every 12-h interval, their genomic DNA was extracted and the ingested fractions of chloroplast and nuclear were quantified by quantitative PCR (qPCR) using *rubisco* and *phytoene desaturase* as respective marker genes. *F. occidentalis*, *F. intonsa* and *T. urticae* showed rapid increasing patterns for the first 6-12 h and then steady patterns over time in their ingested amounts of the chloroplast fractions. The ingested amounts of chloroplasts compared to those of nuclei were 10.0 - 28.1 and 353-fold greater in the two thrips and mite species, respectively; however, the fold differences in ingested amount between these two fractions were substantially lower compared with their original ratio in the kidney bean leaf cells, suggesting that these sucking pests ingest nuclei more selectively compared with chloroplasts. In addition, the number of cells consumed by these thrips and mite species were estimated. Contrast to the cell-feeding insects, neither chloroplast nor nuclear fractions were detected in *N. lugens*, which indicates that *N. lugens* does not ingest any subcellular fractions during sucking. Our findings further suggest that transgenic expression of foreign hairpin RNA in the nuclear would deliver a substantial amount of target molecules to these cell-feeding sucking pests but not likely to the sap-feeding pests when employing ingestion RNAi-based control strategy.

Keywords: Thrips, Sucking pest, cell-feeder, RNAi-based transgenic plant

[P3.18]

Characterization and biotoxicity of biogenic silver nanoparticles synthesized by *Aspergillus niger* as potential eco-friendly control tool against insect pest, *Dysdercus koenigii* (Heteroptera: Pyrrhocoridae)

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Introduction: Presently, the world is facing two important challenges comprising food production and disease control, which put adverse effect on humanity. Eco-friendly control tools against agricultural pests are urgently needed. Silver nanoparticles are widely used in our daily life, mostly due to their antibacterial, antiviral, and antifungal properties. However, their potential toxicity against insect pest remains unclear. **Methods:** In the present work, the biogenic silver nanoparticles were synthesized by fungal biomass of *Aspergilllus niger* and its biotoxicity was evaluated against insect pest, *Dysdercus koenigii.* **Results:** The bioactive volatile compounds, present in the extract, worked as the reducing and stabilizing agent during green synthesis. Further, the formation of silver nanoparticles were confirmed by surface plasmon resonance band illustrated in UV-VIS spectrophotometer. These were further characterized by FTIR, SEM, TEM, EDX and AFM analyses. The characterization studies confirmed the spherical shape and size (15- 24 nm) of silver nanoparticles. Furthermore,. Significant mortality was observed in the insects treated with said nanoparticles. Moreover, results of the cytotoxicity assay showed that the silver nanoparticles also compromised the immune response thereby causing the decrease in the hemocyte viability and total hemocyte count in the treated insects. Silver nanoparticle induced apoptosis was also studied using acridine orange/ ethidium bromide dual staining. In addition, the oxidative stress caused by AgNPs was assessed using enzymes such as superoxide dismutase, glutathione-S-transferase, catalase, and cytochrome p450. **Discussion:** The results of the present investigations might add knowledge on the toxicity of green-synthesized silver nanoparticles, which could be used against agricultural insect pests as a safe bioinsecticide. It would allow us to propose the tested products as effective candidates to develop newer and cheap pest control tools.

Keywords: Silver nanoparticle, Aspergillus niger, Dysdercus koenigii, Biotoxicity

[P3.19]

Gamma-ray Irradiation Control of Whiteflies *Bemisia tabaci* and *Trialeurodes vaporariorum* (Hemiptera: Aleyrodidae) in the Exportation of Fresh Strawberries

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Whitefly (Hemiptera: Aleyrodidae) pests, including the sweetpotato whitefly, *Bemisia tabaci*, and the greenhouse whitefly, *Trialeurodes vaporariorum*, are economically important in agriculture. With the annual growth of the domestic fresh fruit export market, various quarantine treatment methods are being used to export strawberries of better quality. The objective of the present study was to evaluate the effects of gamma rays on the development and reproductive sterility of *B. tabaci* and *T. vaporariorum*. In both species, the eggs were completely inhibited from hatching at 50 Gy, and the emergence of 3rd instar nymphs was completely suppressed at 150 Gy. Some adult *B. tabaci* and *T. vaporariorum* spawning occurred at 100 Gy and 70 Gy, respectively; however, at these irradiation levels, F_1 hatchability was completely inhibited. Dosimetry results showed that the penetrating power of gamma ray in the strawberry-filled box was the lowest at the mid-box position. Therefore, *B. tabaci* and *T. vaporariorum* were placed in the middle of the strawberry-filled box and irradiated. A gamma-ray irradiation of 100 Gy suppressed the development and reproduction of eggs and adults in both *B. tabaci* and *T. vaporariorum*. Our data suggest that at least 100 Gy should be used for the control of these two species of whitefly for strawberry export.

Keywords: Strawberry, Exportation, Gamma ray, Phytosanitary

[P3.20]

Towards understanding common mechanisms of nematode and insect effectors for plant parasitism

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Nematodes and aphids cause substantial yield losses of crops globally. Diversity in their habitats and evolutionary adaptations have led to a remarkable resilience in these pests. Both nematodes and aphids are biotrophic pests, and thus require an intimate association with their host plants to access nutrients. To initiate and maintain parasitism, both groups of pests secrete effectors (proteins or small molecules) through their stylets: these are capable of altering host cell structure and function, evade defences, and enable feeding and development. Sedentary nematodes secrete 'effectors' from esophageal gland cells through their stylet into plant cells, and convert root cells into specialised feeding sites. During probing and feeding aphids secrete watery and gelling saliva, both of which also contain 'effectors'.

To understand whether nematodes and aphids employ similar effectors or mechanisms for parasitizing plants, we have used comparative bioinformatics to identify putative effectors from genomic and transcriptomic data and have extensively characterised candidate proteins *in silico* by predicting common structures, domains and possible localisations in host cells.

So far, fourteen common proteins with similar structures, or with different structures but similar predicted functions, have been identified in both nematodes and aphids, suggesting common mechanisms by which these pests interact with plant hosts. Additionally, *in vitro* feeding with dsRNA and aphid-plant challenge has been carried out.

This insight provides an opportunity to develop a single common control strategy, such as using RNA interference, to silence activity of common essential genes required by both groups of pests for successful parasitism. This approach is economical and sustainable and will contribute to reducing the use of chemical pesticides.

Keywords: Effector Biology, Green Peach Aphid, Pest Control, Plant Parasitic Nematodes

[P3.21]

Quantification of phytoplasma 16SrIX group associated with citrus Huanglongbing symptoms in the leafhopper *Scaphytopius marginelineatus* (Stål)

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Huanglongbing (HLB) is a severe citrus disease associated to phloem-limited bacteria in the genus '*Candidatus* Liberibacter', which were detected in Brazil in 2004. In 2008, another bacterium was found in association with HLB symptom in São Paulo State, and characterized as a phytoplasma belonging to 16SrIX group. Phytoplasmas are vector-borne phytopathogenic mollicutes that inhabit plant sieve elements. This phytoplasma was detected in *Scaphytopius marginelineatus* (Stål), considered a putative vector. The bacteria concentration in the vector body is an indicative of the transmission efficiency of the vector, and this information could be used in prospections of epidemiology and management. The objective of this study was to develop molecular tools to determine the concentration of phytoplasma 16SrIX group associated with citrus Huanglongbing symptoms in *S. marginelineatus*, as well as its relative concentration. Healthy adults from laboratory rearing were caged on phytoplasma PCR-positive citrus plants for an access acquisition period of 72 hours then kept on *Sida rhombifolia* L. plants for a latent period of 21 days. After that, infected leafhoppers were killed, and the total DNA extracted to be used in quantitative Polymerase Chain Reaction (qPCR). The primers to determine the concentration of HLB phytoplasma were designed on the 16Sr bacteria region and to determine the quantity of leafhopper DNA, we used primer designed on 18Sr region of leafhoppers belonging to the same subfamily. The primers used in qPCR showed an efficiency of 114,9% (r^2=0,981) for the phytoplasma and efficiency of 77,3% (r^2=0,981) for insects. The proportion of HLB-phytoplasma genomic units (GU) on *S. marginelineatus* DNA was low (0,7 to 2,22 GU/DNA ng), an indicative this insect could be considered a putative vector, but with very low efficiency. These molecular tools could be applied in plants trials and be used to understand the pathossystem.

Keywords: insect vector, deltocephalinae, qPCR

[P3.22]

Genetic variability and population dynamics of Drosophila suzukii in Germany

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Native to Asia, *Drosophila suzukii* or the Spotted Wing Drosophila (SWD) rapidly became a severe invasive pest in America and Europe. Starting its invasion in Spain and Italy, SWD now belongs to the commonly found Drosophilids in many European countries including France, Switzerland, and Germany, indicating that there is a high potential of ongoing invasion in this species. In contrast to the vinegar fly *Drosophila melanogaster*, SWD is a crop pest of soft-skinned fruits like cherries, blackberries, strawberries, and others. Female flies possess a serrated ovipositor with which they pierce the skin of ripening fruits. Hatching larvae feed on fruit pulp and make the fruits unmarketable, causing a substantial economic loss. Besides the visible damage, fruit skin injuries can entail several other problems like fungal infections. To control this invasive pest species, an understanding of its population dynamics and structure is necessary.

Our study reports the population genetics and development of *Drosophila suzukii* in Germany by using microsatellite markers over different sample sites. As far as known it is the first study that observes changes over three years to discuss the associated consequences for pest control. Early results indicate that SWD populations in Germany show high genetic diversity with differences between southern and northern populations. Additional samples from France, Italy, and Japan are planned to depict if a single or multiple invasions took place. High genetic variability and migration between populations comprise the possibility of fast adaptation to changing environments. This holds not only a high-risk of an ongoing spread of this invasive species but also a high potential of developing pesticide resistance which would be problematic in the future. Either way, there is an urgent need for alternative pest control techniques besides the traditional and in case of SWD often not applicable use of pesticides.

Keywords: Spotted wing drosophila, Microsatellite markers, Population structure

[P3.23]

Understanding the molecular consequences of DDT resistance through multilayered molecular analyses of the 91-R and 91-C *Drosophila melanogaster* fly lines.

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The 91-R and 91-C *Drosophila melanogaster* fly lines represent a unique tool to understand the molecular consequences of DDT resistance. A population of flies, collected from the field over a half-century ago, were separated into two fly lines. The first line, 91-R received decades of DDT selection and the second line, 91-C, is the control population (with no pesticide selection). Within insect systems, these represent a relatively unique resource to study the long-term impacts of pesticide selection on a pair of insect populations of common origin. With the emergence of genomic, metabolic and proteomic tools, the consequences of such prolonged DDT selection can be studies across multiple molecular pathways. We have recently performed genome, and transcriptome sequencing of both of these fly lines and these datasets have been used to study not only phase I, II, and III detoxification genes, but also posttranscriptional control of these gene expression by microRNAs, as well as evolutionary conserved energy-related pathways. Additionally, a selective sweep analysis has led to the discovery of candidate genes for testing towards their direct involvement in DDT resistance. These combined strategies have led to systems-scale analyses that have provided unique insights into pathways that have the potential to change how we approach the management of pesticide resistance. We discuss the possibility for new strategies of how to minimize pesticide resistance in insect populations based on the outcomes of these studies.

Keywords: pesticide, resistance, evolution, genomics

[P3.24]

AgriVectors: a systems biology portal for plant pathosystems and arthropod vectors of plant diseases

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Arthropod vectors of pathogens cause enormous economic losses and are a fundamental challenge for sustainable increases in food production, yet agricultural pathosystems remain an underserved area of research. To more effectively fight plant diseases, data pertaining to a disease system needs to be consolidated, made searchable and amenable to data mining.

The AgriVectors platform is an open access and comprehensive resource for growers, researchers and industry working on plant pathogens and pathosystems spread by arthropod vectors.

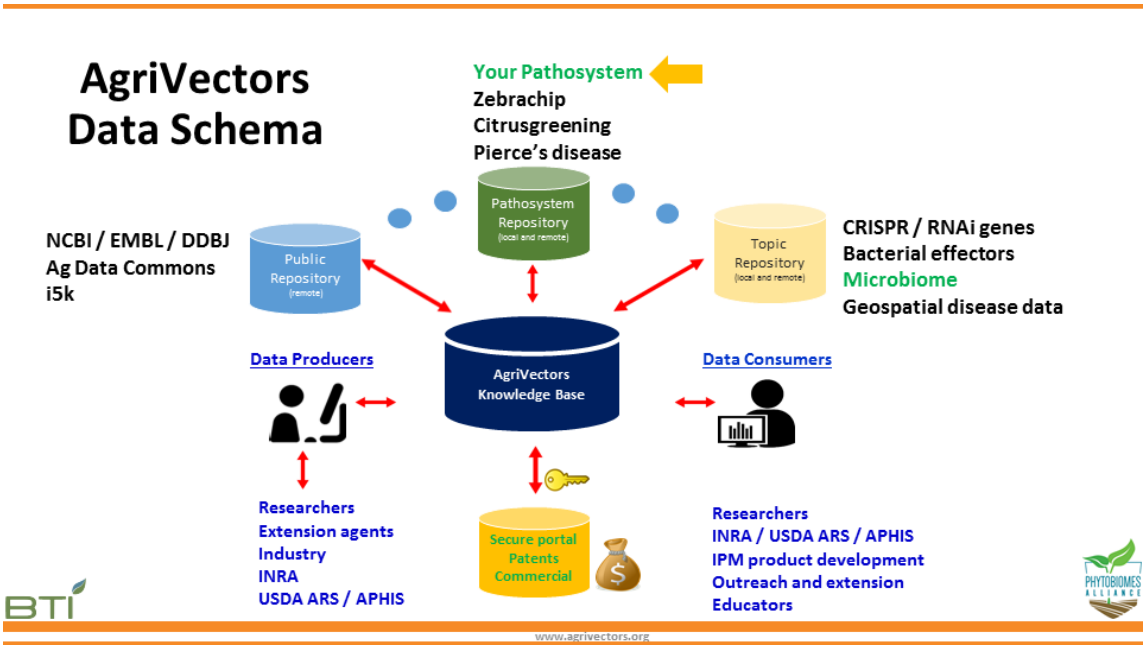

Figure 1: Data schema for integrating information from public, private and topical repositories into the AgriVectors platform

The portal connects established public repositories with pathosystem-specific data repositories. The AgriVectors system will provide tools to enable technologies such as RNAi, CRISPR, screening bioassays, etc. to leverage current and emerging knowledge across disciplines. It will also include private and unpublished data, using passwords and secure protocols for restricted access. The portal will be based on the Citrusgreening.org (https://citrusgreening.org/) community resource that was developed as a model for systems biology of tritrophic disease complexes. Citrusgreening.org provides omics and biology resources for the Huanglongbing pathosystem. In addition, it includes a biochemical pathway database for each organism in this disease complex, and an expression atlas with proteomics and RNAseq data from psyllids (http://pen.citrusgreening.org) and citrus (http://cen.citrusgreening.org) across multiple infection states. The AgriVectors portal will extend this model beyond gene-centric omics data to the broader Pathosystem-wide information, with integrated pest management, behavioural, plant health, soil health and climate data to incorporate rapid phenotyping information from research trials, building a foundation for more effectively identifying solutions to combat plant diseases.

Keywords: Database, Vector, Agriculture, Disease

[P3.25]

Functional validation of resistance mechanisms against sodium channel blocker insecticides via molecular modeling and genome engineering in Drosophila

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Sodium Channel Blocker Insecticides (i.e. Indoxacarb and Metaflumizone) are chemical compounds used as larvicides for the control of a variety of insects such as moths, beetles and flies among other pests. Resistance against this category of insecticides has been identified in the field for several lepidopteran species such as *Tuta absoluta* and *Plutella xylostela*, involving both target site and metabolic resistance mechanisms. In particular the F1845Y and V1848I mutations in the S6 segment of the forth domain have been identified in resistant populations of both aforementioned pests and associated to resistance by *in vitro* studies and molecular modeling, but no *in vivo* validation has been provided to date.

To elaborate their contribution to resistance *in vivo*, we employed genome engineering in *Drosophila melanogaster* via CRISPR/Cas9 coupled with Homologous Directed Repair to introduce these mutations in the fly voltage-gated sodium channel gene.

Toxicity bioassays (oral administration) indicated that both F1845Y and V1848I mutations confer low to moderate levels of resistance (RR: 10.2X and 6X respectively) to indoxacarb; furthermore, V1848I confers similar resistance to metaflumizone (RR: 8.4X), while on the other hand F1845Y has been shown to provide higher levels of metaflumizone resistance by several orders of magnitude (RR: 3441.2X). Molecular modeling of wild type and F1845Y sodium channels suggests that metaflumizone resistance may be attributed to a steric clash between the target site and the 4-cyanophenyl of the insecticide.

Keywords: Sodium Channels, Insecticide Resistance, CRISPR/Cas9, Molecular Modeling

[P3.26]

Is the adipokinetic hormone receptor of the desert locust, *Schistocerca gregaria,* a candidate target for pest control?

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Locust swarms threaten the livelihood of people living in the world's poorest countries by soiling and devouring crops and harvests. Unfortunately, neurotoxic insecticides with limited selectivity are still very commonly employed to combat these insects. In order to improve protection of non-target organisms in the environment and to avoid insecticidal resistance problems, there is an urgent and continuous need for novel, more selective control agents. During energy-demanding processes, such as long distance flight, adipokinetic hormone (AKH) will be released by the *corpora cardiaca*, thereby triggering the mobilization of energy-rich substrates from the fat body. AKH acts by binding to a rhodopsin-like G protein-coupled receptor, which is evolutionarily related to the vertebrate gonadotropin-releasing hormone receptors. A comparative analysis of activities induced by different insect AKH peptides at a variety of AKH receptors covering a wide phylogenetic range of insect species revealed remarkable differences in agonistic selectivity. Furthermore, functional knockdown effects of the AKH signaling system in the desert locust, *S. gregaria*, underscore its future potential as candidate target for pest control.

Acknowledgements: We gratefully acknowledge the EU (Horizon-2020 project nEUROSTRESSPEP), the Research Foundation of Flanders (FWO-Flanders) and the Special Research Fund of KU Leuven (C14/15/050) for financial support.

Keywords: Adipokinetic hormone, Pest Control, Energy metabolism, GPCR

[P3.27]

Functional characterization and high-throughput substrate screening of UDP-glycosyltransferases in a polyphagous arthropod

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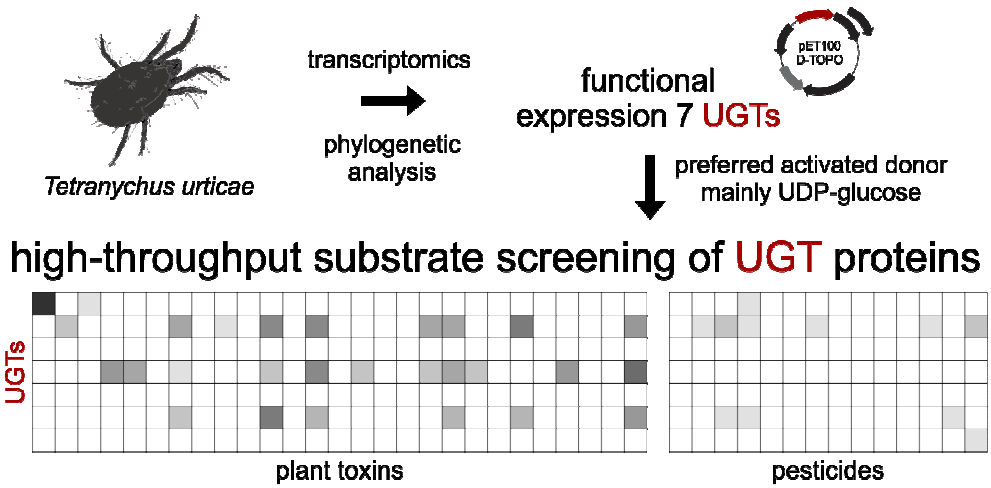
Uridine diphosphate (UDP)-glycosyltransferases (UGTs) catalyze the addition of UDP-sugars to small hydrophobic molecules, turning them into more water-soluble metabolites. While their role in detoxification is well documented for vertebrates, arthropod UGTs have only recently been specifically linked to the detoxification and sequestration of plant toxins and pesticides. The two-spotted spider mite *Tetranychus urticae* (Arthropoda: Chelicerata: Acari) is a generalist herbivore that can develop resistance to pesticides very rapidly. The genome of *T. urticae* harbors 80 UGT genes, which are most likely acquired from bacteria through horizontal gene transfer. This study investigates the potential role of *T. urticae* UGTs in detoxification of both plant toxins as well as pesticides.

A set of seven *T. urticae* UGT enzymes was selected based on their transcriptomic profile upon long-term acclimation to new host plants and/or in mite strains highly resistant to pesticides. In contrast to other arthropod UGTs, *T. urticae* UGTs are cytosolic and can be functionally expressed using *Escherichia coli*. Kinetic activity of the recombinant enzymes was determined, as well as their preferred activated donor for conjugation of model substrates. Subsequently, a high-throughput substrate screening comprising both plant secondary metabolites and pesticides was performed; this led to the selection of nine enzyme-substrate combinations for which the kinetic parameters were further characterized in depth.

All of the recombinant enzymes showed to be catalytically active and the majority of them preferred UDP-glucose as the activated donor for glycosylation. Among others, the acaricide abamectin, and the plant metabolites capsaicin (capsaicinoid), DIMBOA (benzoxazinoid) and kaempferol (isoflavonoid) were shown to be glycosylated.

Our study corroborates the proposed role of *T. urticae* UGTs in detoxification of both synthetic and natural xenobiotic compounds and paves the way for rapid substrate screening of invertebrate UGTs.



Keywords: UDP-glycosyltransferase, pesticide resistance, host plant adaptation, horizontal gene transfer

[P3.28]

Diverse effect of *Solanum nigrum* extract on the toxicity of fenitrothion to *Tenebrio molitor* larvae, due to different strategies of application

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Solanum nigrum extract may increase the toxicity of fenitrothion – a synthetic insecticide, using the appropriate method of application in the *Tenebrio molitor* larvae

Synthetic insecticides are widely used for pest management. In recent years many of their alternatives appeared, such as plant-derived substances. In our research, we focused on the effects of *Solanum nigrum* berries extract on the physiology of the *Tenebrio molitor* larvae. The extract added into the diet of larvae showed sublethal effects on the ultrastructure of the fat body and the midgut cells and changes in the lipids and glycogen level in the fat body. The observed effects were a starting point for our next research, to check the influence of the extract on the toxicity of fenitrothion - a synthetic pesticide, a representative of the organophosphates, which is still commonly used in many countries, e.g. in magazines with stored food, where *T. molitor* is a common pest. *S. nigrum* extract was obtained from prof. Sabino Bufo, Basilicata University, Potenza, Italy. Larvae have been kept separately for 3 days and fed with a nourishment containing examined substances. We conducted our experiments in two variants. In the first one, we mixed with fenitrothion in a concentration that caused medium lethality of larvae with one of 4 tested concentrations of the extract. We did not observe any significant changes in lethality. However, we observed ultrastructural changes in the fat body cells. In the second variant, we gave the extract in one of the 4 concentrations as a preceding factor in the first day of treatment, and fenitrothion in the next two days. We observed increased mortality of larvae. The results showed that the method of use of plant derivatives may increase the toxicity of synthetic pesticides and therefore, contribute to decreasing of their emission to the environment.

Keywords: Solanum nigrum extract, Tenebrio molitor larvae, fenitrothion, toxicity

[P3.29]

Role of arginine kinase in retardation of Spodoptera litura larval growth and development upon feeding with purified T9BBI from Vigna mungo (cv.T9)

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Proteinase inhibitors (T9BBI) from mature dry seeds of *Vigna mungo* (cv.T9) were purified by using ammonium sulphate precipitation followed by affinity and gel filtration chromatography techniques which resulted in ~67.8 fold purification and 33% yield. T9BBI showed a peak with m/z 8209 in Matrix-Assisted Laser Desorption Ionisation Time-of-Flight (MALDI-TOF) mass spectrum correlating to a single band with molecular mass of ~8 kDa in Tricine-SDS-PAGE under non-reducing conditions. The presence of both trypsin and chymotrypsin inhibitory activities together with low molecular mass confirm the purified protein as Bowman-Birk inhibitor. Also, the T9BBI showed significant inhibitory activity against trypsin-like mid-gut proteases of *Spodoptera litura* 6^{th} instar larvae. Feeding of *S. litura* 2^{nd} instar larvae on diet supplemented with T9BBI showed a dose-dependent decrease in body weight of larvae and pupae, formation of larval-pupal as well as pupal-adult intermediates and delay in pupal development as compared to larvae fed upon control diet. Separation of midgut extracts by 2-D gel electrophoresis and analysis of protein spots using Image master 2D platinum 7.0 software identified a total of 110 spots, among which the expression of 33 spots were up-regulated (T/C $\geq$1.5) and 13 were down-regulated (C/T$\geq$1.5), while two of them were found new in treated samples as compared to control. MALDI-MS/MS analysis of five 2-D protein spots with significant changes showed matching with zinc finger protein (down-regulated), arginine kinase isoform-3 (down-regulated), myosin heavy chain (new), actin muscle type A2 (up-regulated) and actin 5c (up-regulated) in NCBI database. Furthermore, corroborating with protein levels in 2-D gel, the activity of arginine kinase decreased in larvae fed upon T9BBI as compared to control. Taken together, these results suggest T9BBI inhibits the growth and development of *S. larvae* by modulating the amount and activity of arginine kinase present in midgut tissues

Keywords: Spodoptera litura, MALDI-TOF/TOF, Two-dimensional gel electrophoresis, Arginine kinase

[P3.30]

Improved RNAi effects in fruit flies by exploiting an engineered insect virus as a delivery vehicle

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Introduction: The spotted wing Drosophila (*Drosophila suzukii*) is an invasive and serious economic pest to small and stone fruits and its control is difficult. RNA interference (RNAi) or double stranded RNA (dsRNA)-mediated gene silencing is rapidly becoming a widely used functional genomics tool in insects and holds great potential for insect pest control. However, the delivery of dsRNA is a challenging step in the development of RNAi bioassays. In this study, we investigated the possibility of exploiting a recombinant virus for efficient and specific delivery of dsRNA to fruit flies.

Methods: With a practical example, we demonstrate how the RNA virus, Flock House Virus (FHV; Nodaviridae), can be engineered to induce targeted gene suppression through RNAi under both in vitro and in vivo conditions. As proxy for fruit flies of agricultural importance, we worked with S2 cells derived from *Drosophila melanogaster* embryos, and with adult stages of both *D. melanogaster* and *D. suzukii*.

Results: We found that the expression level for all of the targeted genes were reduced by more than 70% in both the *in vitro* and *in vivo* bioassays. Furthermore, cell viability and median survival time bioassays demonstrated that the recombinant FHV expressing target gene sequences caused a significantly higher mortality than the wild type virus, in both S2 cells and adult fruit flies, respectively.

Discussion: This is the first report showing that a single stranded RNA insect virus such as FHV, can be engineered as an effective *in vitro* and *in vivo* RNAi delivery system. Since FHV infects many insect species, the described method could be exploited to improve the efficiency of dsRNA delivery for RNAi-related studies in both FHV susceptible insect cell lines and live insects that are recalcitrant to the uptake of naked dsRNA.

Keywords: RNAi, Drosophila suzukii, Flock house virus, double stranded RNA

[P3.31]

Detection of plant pathogens on insect vectors in Galicia (NW Spain) by molecular techniques

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The agricultural and forestry sectors represent an important part of the economy of Galicia (NW Spain). Diseases caused by plant pathogenic fungi such as *Fusarium circinatum* (reported in Galicia in 2004), bacteria such as *Candidatus* Liberibacter spp., and phytoplasmas such as *Candidatus* Phytoplasma vitis (both bacteria and phytoplasma not yet detected in the region) produce significant economic losses in these sectors and seriously affect the viability of the plants they may damage. Insect vectors can disseminate propagules of pests to non-affected areas and infect new hosts. The identification of phytopathogenic organisms in diagnostic laboratories can be done directly from insect vectors by the application of molecular techniques.

The aim of this work was to detect *Fusarium circinatum*, *Candidatus* Liberibacter spp., and *Candidatus* Phytoplasma vitis, which are pathogens on *Pinus* spp., *Citrus* spp., and *Vitis* spp., respectively, on insects from Galicia using molecular techniques. For the detection of *F. circinatum*, responsible for pine resinous canker, 221 specimens of *Ips sexdentatus*, 40 *Hylurgus ligniperda*, 16 *Spondylis buprestoides*, 15 *Temnochila caerulea*, 11 *Thanasimus formicarius*, 5 *Orthotomicus erosus*, and 2 *Hylastes attenuatus* were captured in forests of *Pinus* spp. and analyzed by real-time PCR and DNA sequencing. For the diagnostic of *Candidatus* Liberibacter spp., which causes the disease called Huanglongbing, 60 specimens of *Trioza erytreae* were studied by real-time PCR. Twenty specimens of *Scaphoideus titanus* were examined by multiplex nested PCR for the study of *Candidatus* Phytoplasma vitis, responsible for grapevine flavescence dorée.

The presence of *F. circinatum* was recorded in individuals of *Ips sexdentatus*, *Orthotomicus erosus*, *Temnochila caerulea* and *Thanasimus formicarius* whereas *Candidatus* Liberibacter spp. and *Candidatus* Phytoplasma vitis were not detected in any of the studied specimens.

These results indicate the need to continue with this research to assess the action of insect vectors in the dissemination of important diseases.

Keywords: disease, insect vector, molecular techniques, detection

[P3.32]

Piperidine derivative as larvicidal agent against *Aedes aegypti* (Diptera: Culicidae) and cholinergic inhibitor

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Aedes aegypti spread viral diseases, such as Dengue, Zika and Chikungunya. Vector control is necessary to reduce and avoid pandemics; to achieve this goal, insecticides are used. Most insecticides are acetylcholinesterase (AChE) inhibitors, like some piperidine derivatives.

Thus, this work was focus on analyze larvicidal activity of 4-allyl-4-N-(4-methylphenyl)amine-1-bencylpiperidine against a field strain of *Aedes aegypti* (Piedecuesta, Colombia) using WHO protocol; evaluate the cholinergic effect of this compound over larvae after different times of exposure utilizing Ellman colorimetric method; and determine the molecular insight of the cholinergic inhibition by docking simulations working with a 3D stable structure of the *Aedes aegypti* acetylcholinesterase (aaAChE) obtained by threading of sequence fragments (code Q6A2E2) and equilibration after 180ns of molecular dynamics simulations.

The results showed that 4-allyl-4-N-(4-methylphenyl)amine-1-bencylpiperidine is highly active against *Ae. aegypti* larvae (CL_{50} 32.9±0.1µg/mL). Moreover, the cholinergic system of *Ae. aegypti* larvae was destabilized by the compound, after 2 hours of exposure it was observed a reduction of 64.6% on the cholinesterase (ChE) level (8.5mU ChE; control 12.4mU ChE); 12 hours of exposure was noticed an increase of the amount of cholinesterases, then the inhibitory effect was only 6.4% (13.7mU ChE; control 14.6mU ChE); finally, after 48 hours of exposure this piperidine promoted the maximum cholinergic inhibition (87.6%; 2.7mU ChE; control 21.5mU ChE).

Molecular docking results shown some possible interactions between the allyl-bencylpiperidine and the residues from the catalytic triad in the active site of aaAChE (Glu325, Ser326 and His566), with a low probability of being a spontaneous process (ΔG -0.8kcal/mol), even though this molecule can form a hydrogen bond with Gly567 (residue in the active site).

These findings suggest that piperidine derivates like 4-allyl-4-N-(4-methylphenyl)amine-1-bencylpiperidine could be considered as a new kind of larvicide to control the *Aedes aegypti* vector and its possible mechanism of action is the competitive/mixed inhibition of acetylcholinesterase.

Keywords: Aedes aegypti, Cholinergic inhibitor, Larvicidal activity, Piperidine derivative

[P3.33]

Citrus sinensis, C. reticulata and C. limon essential oils as larvicides against two Colombian resistant strain of Aedes aegypti (Diptera:Culicidae)

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The peels of the *citrus* genus are been used as renewable sources of phytochemicals with a wide variety of activities in which the antioxidant, bactericidal, fungicidal and insecticidal are remarkable.

In this work, three essential oils (EO) from *Citrus sinensis*, *Citrus reticulata* and *Citrus limon* were obtained by microwave hydrodistillation and characterized by gas chromatography-mass spectrometry. The EO were evaluated as larvicides over three strains of *Aedes aegypti*; one susceptible (Rockefeller) and two field collected (Piedecuesta and Bucaramanga cities), using the WHO protocols; the resistance to commercial insecticide and EO of the two field strains was also determined. Additionally, the inhibition of the acetylcholinesterase was studied.

The highest EO extraction yield was obtained with *Citrus limon* (0.30%), flowed by *Citrus sinensis* (0.20%) and *Citrus reticulata* (0.15%). Limonene was one of the most abundant compounds of EO - *C. sinensis* (74.45%), *C. reticulata* (75.09%) and *C. limon* (27.76%). All the EO were highly active (LC_{50} < 50 µg/mL) against mosquitoes' larvae, with lethal concentrations between 16.95±0.31 and 33.91±0.69 µg/mL. Both field strains were susceptible (RR_{50} > 5) to these EO, showing resistance ratios between 0.86 and 1.07. However, Piedecuesta and Bucaramanga strains were resistant (RR_{50} > 10) to chlorpyrifos (Piedecuesta 15.08, Bucaramanga 11.21) and malathion (Piedecuesta 13.85, Bucaramanga 28.21). The inhibition of the acetylcholinesterase showed that the EO should be considered as weak inhibitors of this enzyme (IC_{50}>150 ppm); *C. sinensis* EO (150.5±2.7 ppm) was the most active inhibitor, followed by *C. limon* EO (158.1±3.4 ppm) and *C. reticulata* EO (232.8±15.5 ppm).

These EO showed to be highly activity, with low resistant, against third instar larvae of *Aedes aegypti*, as well a low toxicity over the acetylcholinesterase. Furthermore, results suggest these essential oils as new strategy to control field strains of *Aedes aegypti*.

Keywords: acetylcholinesterase inhibition, Aedes aegypti, Citrus essential oils, Larvicidal activity

[P3.34]

Molecular basis of olfactory-driven behaviors of *Culicoides imicola*

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Culicoides imicola (Diptera: Ceratopogonidae) is a highly successful biting midge, infamous for its role as a biological vector for numerous pathogens of veterinary significance. Like in most insects, olfaction is likely to play a central role in the long-range attraction of *Culicoides* towards animal-hosts, oviposition sites and sugar sources. However, studies of their sensory apparatus are scarce and the vast majority of semiochemicals used by *Culicoides* are unknown. Our tanscriptome analysis show that *C. imicola* exhibits many members of the odorant, ionotropic and gustatory receptor gene families. Using behavioral experiments, we also establish the basic attractiveness to several odorants. This novel, basic information of the mechanisms governing *C. imicola* long-range attraction to nutritional sources and oviposition sites that may be of major agricultural significance and may find its way in a number of applications such as new monitoring and or biocontrol strategies aiming at the destabilization of these notorious veterinary pests.

Keywords: Culicoides imicola, long-range attraction, olfaction

[P3.35]

Genome wide association study reveals two new loci associated with pyrethroid resistance in *Aedes aegypti*

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Introduction: A few mutations in the voltage gated sodium channel (Na_v) have been associated with pyrethroid resistance in *Aedes aegypti*. Although point mutations are related to the level of knockdown resistance little is known about other loci contributing to metabolic resistance. We carried out a genome wide association study (GWAS) with two pyrethroid resistant populations of *Ae. aegypti* and identified, for the first time, two new loci associated with the pyrethroid resistance not in the Na_v gene.

Methods: Mosquitoes were collected in Macapa and Oiapoque, Amapa, Brazil, in 2015 using 60 ovitraps over a period of 3 weeks. Eggs were hatched and reared in standard conditions. We exposed 5 days old female mosquitoes to deltamethrin following the World Health Organization guidelines for test tube bioassays. We classified mosquitoes from both populations into 3 phenotypes: resistant, susceptible, and with knockdown resistance. We extracted genomic DNA from all individuals and genotyped 95 using Axion aegypti1 50K SNP chip. We conduct the GWAS treating the trait as continuous, tested different effects, statistical models, and did adjustment for multiple testing.

Results: Mosquitoes from Oiapoque are more resistant to deltamethrin. We exposed them to a higher dose (papers treated with1,200mg/L) and 76% were resistant, 1% displayed knockdown resistance and 22% were susceptible. Meanwhile Macapa were exposed to a lower dose (600mg/L) and only 17% were resistant, 9% displayed knockdown resistance and 74% were susceptible. The GWAS revealed two SNPs associated with the insecticide resistance. AX-93253438 on chromosome 2 and AX-93227955 on chromosome 3.

Discussion: We discovered two intronic SNPs associated with the pyrethroid resistance and they are not on the Na_v gene. The nearest gene to AX-93253438 is AAEL011338 with unknown function. The nearest gene to AX-93227955 is AAEL003896 which is a serine kinase. These genes may contribute to pyrethroid resistance metabolizing the insecticide.

Keywords: Gwas, pyrethroid, metabolic, kdr

[P3.36]

Metamorphosis of Ixodes ricinus (Acari: Ixodidae) causes a shift in structure and functions of bacterial community

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Ixodes ricinus is one of the most common European tick species transmitting infectious agents to both human and animal hosts during blood-feeding. Tick metamorphosis causes significant alteration in the nutrimental, developmental and ecological conditions of ticks; however, the impact of these changes on the tick microbiome including pathogen members is a gap in knowledge. In this study, field-collected I. ricinus individuals were sequenced by Illumina MiSeq 16S amplicon analysis across three extraction approaches. Taxonomic annotation showed that moulting from nymphs to adult female caused a decrease in the alpha diversity of the bacterial community and relative abundances of phyla Actinobacteria and Firmicutes, while an increase was observed in the relative abundance of Proteobacteria. Hierarchical clustering with heatmap at class level and principal components analysis at family level also showed a strong separation in composition of bacterial communities between nymph and female ticks. Our findings at genus level demonstrated that I. ricinus nymphs harboured unique and more diverse bacterial community including pathogen members of genera Anaplasma, Borrelia, Candidatus Neoehrlichia, Rickettsia and Spiroplasma, while Rickettsia monacensis and Candidatus Midichloria mitochondrii as a clonizer partner bloomed within I. ricinus females. Functional profiling related that metabolic functions in bacterial communities were more abundant in nymphs than in adult females. Our study pointed out that the metamorphosis associated with developmental, nutrimental and/or environmental changes caused a significant alteration in their structure within ticks and this could affect the pathogen occurrence. This study also highlighted that I. ricinus females might reserve the metabolic and secretory processes in order to conserve resources and this also cause a reduction in the metabolic activities of bacterial communities during fasting period after metamorphosis.

Keywords: castor bean tick, metabolic function, tick microbiome, pathogens

[P3.37]

Amino acid substitutions and intron polymorphisms of voltage-gatedsodium channel associated with pyrethroid resistance of *Aedes aegypti* in Southern Taiwan

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Aedes aegypti is the main vector of Dengue fever in Southern Taiwan. It has been controlled by pyrethroids for more than three decades and developed relatively high resistance to these insecticides. In this study, *Ae. aegypti* collected from different districts of Kaohsiung and Tainan City were tested for the resistance development to various pyrethroids, the frequency of amino acid substitutions, e.g. S996P, V1023G, F1565C and D1794Y, of voltage gated sodium channel (VGSC) and the association between the mutation frequency of VGSC and pyrethroid resistance in these field strains. The adult knockdown bioassay has shown that relatively high knockdown resistance was detected in both Kaohsiung and Tainan *Ae. aegypti* against permethrin, cypermethrin and fenvalerate, >50 fold in average, while less resistance was found against □-cypermethrin, deltamethrin, □-cyholothrin, cyfluthrin and etofenprox, <35 fold in average. From PCR/RFLP analysis, four mutant haplotypes and eight diplotypes, while no $S_{(intron\ A)}$VFD or $S_{(intron\ B)}$VFD wild haplotype, were detected in these field strains. After analyzing with IBM SPSS statistics 20.0 and Spearman's rank correlation coefficient, another mutant haplotype was found to be associated with permethrin resistance, one associated with resistance of type II pyrethroids, and one negatively correlated with fenvalerate resistance. In summary, S996P is significantly associated with resistance against all tested pyrethroids except permethrin, fenvalerate and etofenprox; V1023G is significantly associated with resistance against all tested pyrethroids except □-cypermethrin and etofenprox; and D1794Y is only significantly associated with permethrin resistance.

Keywords: Aedes aegypti, knockdown resistance, amino acid substitution, voltage-gated sodium channel

[P3.38]

Epidemiology of pediculosis in southwestern Iran (2008-2013)

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Introduction: Head lice infestation affects millions of humans each year, particularly children of 5-14 years of entire socioeconomic categories. *Pediculus capitis* especially infest persons with poor hygiene, and it is an important challenge among the vagabond people and in refugee camping sites. The present study was conducted in order to determine the epidemiology of *Pediculus capitis* from 2008 to 2013 in eastern areas of Ahvaz County, southwestern Iran.

Methods: The gathered head lice were transferred into glass bottles containing 70% alcohol. The hair and scalp of each person was examined for lice or nits by a trained examiner under the supervision of the principal investigator. The age, place of residence, month, sex, history on infestation of the host were recorded. The analysis was performed using SPSS version 18.

Results: A total of 5446 infected cases were detected, that 72.1% of them resided in urban areas. The prevalence of head lice was highest (41.2%) in age group of 6-10 years and lowest (6.9%) in age group of less than six years. The majority of cases (49.7%) were detected in winter. Statistically significant relationships were found between head lice infestation, and factors such as residency status, season, and age groups ($P<0.05$). The prevalence of infestation was significantly higher in girls (94.4%) than in boys 5.6 % ($P<0.05$).

Conclusion: We found a high prevalence rate of head lice infestation in this study. Gender, age group, season and a history of contact with an infected person were the main modifiable risk factors.

Keywords: Trend, Epidemiology, Incidence, Head Lice

[P3.39]

Infection phenotype analysis of the *Dirofilaria immitis* in *Aedes aegypti*-infected with *Wolbachia pipientis*

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In recent years, mosquito-borne infectious diseases such as dengue fever have become a big problem and the use of symbiotic *Wolbachia*-infected mosquito that does not transmit dengue virus is focused. The social implementation experiments of *Wolbachia*-infected mosquito are being promoted in various parts of the world. However, it is unclear whether a *Wolbachia*-infected mosquito is useful against parasitic nematode infection such as filariasis or has a negative effect. Therefore, we tried to determine whether *Wolbachia*-infected mosquito could be a useful tool against filariasis or not. We used dog heartworm as a model of mosquito-born filariasis. Dog heartworm disease is caused by *Dirofilaria immitis* and is one of the most important lethal parasite diseases among companion animals. Heartworm disease is still endemic in many regions of the world including developed countries such as Japan and Western countries. In this study, by using *Wolbachia pipientis*-infected *Aedes aegypti* and the canine heartworm worm laboratory strains, we analyzed the *D. immitis* infection phenotype at the mosquitoes-infected with*W. pipientis*. In the phenotypic analysis, we analyzed the mosquito survival after *D. immits* infection and the number of infective stage larvae L3 produced in each mosquito. There are no significant differences in survival and L3 number between *W. pipientis*-infected mosquito and control. The L3 isolated from *W. pipientis*-infected mosquito maintained normal developmental ability in in vitro molting assay. These results indicated that *W. pipientis* does not affect to the vectorial capacity of *Ae. aegypti* for *D. immitis* infection.

Keywords: Aedes aegypti, Dirofilaria immitis, Wolbachia pipientis

[P3.40]

Dermacentor nuttalli with Gene polymorphism in Inner Mongolia had potential risk to transmit the human and animal Brucellosis

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Abstract Introduction: *Dermacentor nuttalli* (*D.nuttalli*) are native tick species in Inner Mongolia. Ticks carry a variety of pathogens that can be transmitted to a wide range of animals. The recent outbreaks of brucellosis may be related to the increased activity from ticks and other vectors. The objective of this work was to investigate the relationship between *D.nuttalli* and *Brucella* specific genes in order to assess the potential risks whether *D.nuttalli* transmit *Brucellosis*.

Methods: 1500 tick samples were collected from 15 pastoral areas in Hulun Buir of Inner Mongolia during 2015-2016 and to be identified through the phylogenetic tree analyses of 16S rRNA and ITS2 genes. For brucellosis pathogen detection, DNA was extracted from ticks at different developmental stages and parts of salivary glands and midgut were used to be templates for amplifying of *Brucella* specific genes□BCSP31□by standard and TaqMan Real-Time PCR.

Results: Tick samples were identified as *D.nuttalli*. Different percentage of tick samples were found in *Brucella* specific genes by using the standard PCR in the past two years which made the conclusion that ranged from 6.25% to 87.80%. Later on, each 50 positive and negative samples were randomly selected from two years' samples for the quantification by Real-Time PCR. Consequently, the maximum and minimum copies was 2.44×105 (ct 23.67) and 16.39 (ct 37.07)respectively. In addition, *Brucella* specific genes were successfully detected from all developmental stages and different parts of salivary glands and midgut of ticks.

Discussion: This finding demonstrated *D.nuttalli* is able to carry *Brucella* for a long time and it might transovarial transmit *Brucella* among *D.nuttalli* populations which means that *D.nuttalli* might be a new host for *Brucella*. Therefore, there is a potential risk that *Brucellosis* may be spread through biting animal or even human beings.

Keywords: D.nuttalli, Brucella specific gene, Developmental stages, Salivary glands

[P3.41]

A simple and fast microfluidic system for separation and concentration of sperm and hemocytes in sandfly, Phlebotomus papatasi (Diptera:Psychodidae)

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Phlebotomus papatasi is a major vector of cutaneous leishmaniasis, one of the most important endemic diseases in Iran. Accurate cell sorting and analysis is very important in the related cellular and molecular studies. The structure of hemocytes and sperm of male species of *Phlebotomus papatasi* has not been studied. In this work, we developed a microfluidic system to separate and concentrate the hemocytes and sperm of male *Phlebotomus papatasi* and facilitate the analysis by light and electron microscopy.

To prepare the sample, male sandflies were pooled and mechanically squashed in phosphate-buffered saline. The mixture was then filtered using a paper filter to remove debris. The prepared cell suspension was transferred into a syringe connected to the microfluidic device by appropriate tubing. The microfluidic device was fabricated using soft lithography. It includes an input, a microchannel equipped with microstructures capable of trapping particles of certain size, and an output. The cell suspension was introduced into the device using a syringe pump. As the cells pass through the microchannel, a group of them got trapped into the microstructures in accordance with their dimensions. The other group was transferred into the outlet and collected in a vial for microscopy. The maximum size of the collected cells was determined by the suspension flow rate. The process was repeated in a cascade manner to separate and concentrate variant cells.

As a result, different cells were sorted and separated based on their dimensions for further analysis. The microfluidic system is simple, compact, and portable and can be used widely in the field to study cells at low concentrations. It can be also used as a diagnostic kit for different parasites such as *Leishmania* from infected female sandflies. Our approach paves the way for implementation of new, fast and simple devices to be used in parasitology.

Keywords: Phlebotomus papatasi, Microfluidics, Cell separation

[P3.42]

A twenty- four years study on the malaria trend in southwestern Iran (1995-2018)

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Introduction: Iran has attained more than 96 percent reduce in indigenous malaria cases, and is classified in the elimination phase. Almost all malaria transmission happens in the southeastern regions of the country. Most of autochthonous malaria cases in Iran are due to *Plasmodium vivax*. In Iran, there is a significant decrease in disease burden; however, the overall trend of malaria prevalence is not investigated or well-documented in different localities. Hence, this study is aimed to investigate the epidemiologic features of malaria cases in Gotvand County from 1995 until 2018. **Methods:** This descriptive cross-sectional survey investigates malaria-related factors during a 24-year period of time based on existing data and information extracted at Gotvand's Health Services Center during 1995-2018. Malaria infected cases were confirmed by direct microscopy and treated with normal antimalarial agents. For each positive case a questionnaire containing demographic and epidemiologic data was filled out. Data analysis has been done by SPSS software. **Results:**The obtained data from the reviewed forms included 46 positive cases in Gotvand County. The total number of malaria cases has been decreased in 1995 and 2012 compared to 1998. The highest (23, 5 and 5) cases of malaria were occurred in 1995, 1996 and 2006, respectively. The majority of cases (67.4%) were male. Most cases of malaria were due to *P. vivax* (97.8%) followed by *P. falciparum* (2.2%). About 69.6% of cases were Iranian and 30.4 % non-Iranian (Afghan). Most cases of the disease (32.7%) were in age group of 20-29 years old. The highest number of infections was seen in the occupational group of workers with 19 (41.3%) cases. **Conclusion:** With respect to suitable environmental conditions for transmission of malaria in this area, necessary plans should be conducted in Khuzestan Province to prevent the reoccurrence of malaria in this county.

Keywords: Malaria, Epidemiology, Trend, Iran

[P3.43]

Bioecology of phlebotominae sandflies in Khorramshahr county, southwest of Iran (2017-2018)

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. **Introduction**: Leishmaniasis is one of important tropical diseases caused by *Leishmania* parasites which is transmitted by biting of female phlebotomine sandfies. The main objective of this study was to determine the species richness and relative abundance of sandflies, monthly prevalence, sex ratio and abdominal situation of sand flies in indoor and outdoor resting places in Khorramshahr County, southwestern Iran.

Methods: From April to January 2017-2018, sandflies were collected monthly by 60 sticky traps from outdoor and indoor resting places. In this method, traps were installed after sunset and were collected before the following sunrise. Sand flies were removed from the traps, rinsed in acetone and then conserved in 70% ethanol. All specimens were mounted as temporary or permanent microscopy slides, using Puri's medium.

Results: Sandfies species were *Phlebotomus papatasi, P. alexandri, Sergentomyia sintoni , S. dentate, S. tiberiadis, S.baghdadis,S. iranica, S. theodori, S.antenata, S.squamipleuris* and *S. palestinensis* . Among the species belonging to genus *Phlebotomus* and *Sergentomyia* , *P. papatasi* , *S. sintoni* , *P.alexandri* were the most predominant species, comprising 39.7% , 32.2% and 21.1% of the total number of specimens, respectively. The sex ratio of *P. papatasi*, *P.alexandri* and *S.sintoni* were 222.7 , 414.3 and 27.5, respectively. *P. papatasi* and *S. sintoni* were more abundant in indoors and outdoors, respectively. Sandfly activity started in April and ended in January. Females analysed according to their gonotrophic stage which majority were unfed.

Conclusion: High density of *P. papatasi* as the dominant specimen indicates that, this species can be the main vector of disease. Collection of *P. alexandri* from rodent burrow show that it can play as the secondary role to transmitting of disease among rodent reservoirs. Control of rodents, environmental sanitary as well as personal protections and insecticide impregnated bed nets was suggested to prevention of disease.

Keywords: Fauna, Monthly Activity, Sandfly, Leishmaniasis

[P3.44]

Detection and molecular analysis of acaricide resistance in the poultry red mite Dermanyssus gallinae

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Introduction:

The poultry red mite (PRM), *Dermanyssus gallinae*, is the most important ectoparasite of laying hens, posing major threats to both animal welfare and public health, with an economic impact to EU egg producers of more than 230 million euros in 2017. PRM control is currently based on a limited number of acaricides and consequently resistance developed. However, PRM resistance mechanisms against acaricides have not yet been thoroughly analysed and elucidated at the molecular level.

Methods:

PRM populations across Europe were monitored for the presence and frequency of insecticide resistance phenotypes and resistance alleles, using contact acaricide bioassays as well as molecular assays and sequencing, respectively.

Results:

Moderate to high levels of acaricide resistance were identified in in several PRM populations (examples in Table 1). The most striking resistance phenotypes were analyzed at the molecular level. Mutations in the voltage gated sodium channel were found to be associated with pyrethroid resistance. Their presence and frequency were monitored and found variable across several geographical regions in Europe (Figure 1).

Discussion:

Acaricide resistance was detected and associated with the history of spraying applications in some cases. Novel resistance mechanisms are under investigation. The presence of *kdr* resistance mutations, associated with pyrethroid resistance was detected in *Dermanyssus gallinae* and their frequency was determined. The results and outcome of this work, such as molecular diagnostics for the early and accurate detection of resistance, will help poultry farmers for the application of effective and sustainable PRM management programs.

Table 1 The response of Red mite populations from Greece to insecticides / acaricides of different mode of action

Treatment	Population	LC50 (mg/L)
α-Cypermethrin	Megara(GR1)	>2000
	Liosia (GR2)	>2000
Abamectin	Megara(GR1)	8.45
	Liosia(GR2)	298
Acetamiprid	Megara(GR1)	101
	Liosia(GR2)	210
Pyridaben	Megara(GR1)	7.53
	Liosia(GR2)	272

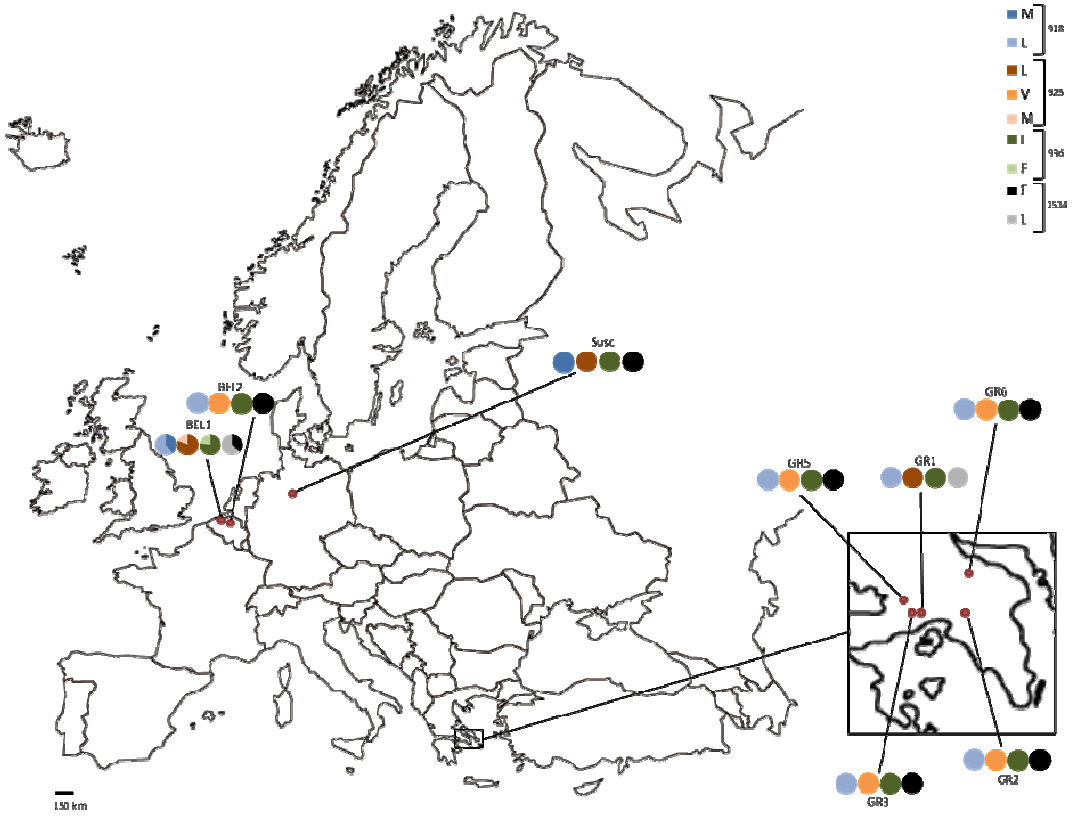

Figure 1 Global distribution and allele frequency of *Dermanyssus gallinae* populations

Keywords: diagnostics, poultry farming, ectoparasite management, resistance

[P3.45]

Functional characterization of the malaria vector *Anopheles gambiae* 4G cytochrome P450s in oenocyte-specific *cyp4g1* knock-down *Drosophila*

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In *Drosophila melanogaster* a cytochrome P450, DmCYP4G1 catalyses the insect-specific P450 oxidative decarbonylase step in CHCs biosynthesis and the oenocyte-specific RNAi-based silencing of *cyp4g1* results in flies that suffer from severe desiccation and eventually die at the time of adult emergence.

In *Anopheles gambiae* two CYP4Gs have been found, CYP4G16 and CYP4G17, both localized in oenocytes. In line with DmCYP4G1, CYP4G16 of *Anopheles gambiae* has also been shown *in vitro* to have a decarbonylase activity, and hence a role in hydrocarbon biosynthesis is postulated, but the actual function of CYP4G17 has not been yet elucidated.

We investigate the role of both CYP4G17 and CYP4G16, alone and/or in combination, by functionally expressing in *Drosophila melanogaster* flies in which the endogenous gene (*cyp4g1*) has been RNAi suppressed in oenocytes. Heterologous expression of CYP4G16, CYP4G17 in one or two copies and their combination revealed different abilities of each protein to rescue the adult lethal phenotype of *cypg1*KD flies. CYP4G16 alone (when in two copies) or in combination with CYP4G17 almost completely restored viability, while CYP4G17 is also capable to confer ability of normal eclosion but less sufficiently. We also performed CHC analysis to the survivors to identify which hydrocarbons can be synthesized by each *An. gambiae* P450 and their combination. We identified interesting differences that will be discussed in line with the intriguing 4G protein localization in different life stages and possible biological relevance.

Keywords: Anopheles gambiae, P450s, oenocytes, Cuticular Hydrocarbons

[P3.46]

Effect of electron beam irradiation on enzyme activity and gene expression in *Culex pipiens* and *Aedes albopictus* (Culicidae: Diptera)

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Enzyme activity and gene expression of sterile mosquitoes using electron beam irradiation.

Previous studies have confirmed the dose (Gy) of sterilization and compared the differences of each mosquito by electron beam treatment with control. Sterile doses of both male and female were 120 Gy for *Cx. pipiens* and 70Gy for *Ae. albopictus*.

Several detoxifying enzyme (GST, non-specific esterase and MFO) activity of two mosquitoes adult were investigated.

In addition, the treatment of electron beam examined the changes in the stress genes and genes on the metabolic function in two species of mosquitoes.

This study was to observe the effect of electron beam on mosquitoes and to investigate the possibility of sterile insect technique (SIT).

Keywords: electron beam irradiation, sterilization, Cx. pipiens, Ae. albopictus

[P3.47]

Resistance-related mutations in the Brazilian Culex quinquefasciatus populations

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Culex quinquefasciatus (Diptera: Culicidae), commonly known as pernilongo, is a mosquito widely distributed throughout Brazilian territory. This anthropophilic species is the main vector of pathogens responsible for the development of diseases such as lymphatic filariasis and West Nile Fever. Environmental management and use of insecticides are the most employed strategies to decrease density of natural populations of this vector. However, the exacerbated use of insecticides has been selecting resistant populations worldwide. The goal of this study was to investigate the diversity of the main genes related to resistance to insecticides in Brazilian populations of *Cx. quinquefasciatus*. To date, PCR-based genotyping methods and DNA sequencing have been employed to evaluate samples from nine localities (Boa Vista /RR, Cáceres /MT, Campina Grande /PB, Caseara /TO, Cuiabá /MT, Foz do Iguaçu /PR, Manaus /AM, Oiapoque /AP and Recife/PE) covering the five regions of the country. The del19 and G1324A mutations in the *cqm1* gene, related to resistance to *Bacillus sphaericus,* were not identify. However, in some of these populations we found the classical L1014F (*kdr* mutation) and G119S (ace-1^R) substitutions in the Na_V and *ace-1* genes, related to pyrethroid and organophosphate resistance, respectively. The presence of alleles that are classically related to resistance in populations of *Cx. quinquefasciatus* from different regions of the country, strongly indicates the need to implement an insecticide resistance monitoring program for this vector.

Keywords: pernilongo, ace-1R, kdr

[P3.48]

CRISPR/Cas9 mutagenesis in *Phlebotomus papatasi*: the sand fly immune response impacts the vector competence for *Leishmania major*

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Introduction: Sand flies are the natural vector for transmission of *Leishmania* parasites. The emergence of the CRISPR/Cas9 technology as a genome editing tool in insects opened new possibilities for studying sand fly / *Leishmania* interactions. For several arthropod vectors, the insect immune response has been shown to impact the development and transmission of the pathogen to its host. The Immune Deficiency (IMD) pathway is one of the two key signaling cascades controlling the immune response in insects. Here, we adapted the CRISPR/Cas9 technology to *Phlebotomus papatasi* sand flies, a natural vector for *Leishmania major*, targeting the gene encoding the IMD pathway transcription factor, Relish (Rel), in order to decipher its contribution to the insect permissivity for *Leishmania*.

Methods: We designed the molecular tools for our CRISPR/Cas9 mutagenesis, established a protocol for sand fly embryo injection and rearing, and used a PCR-screening approach to identify and maintain mutant alleles. The gut microbiota and permissivity to *Leishmania* parasites of sand flies carrying *rel* null alleles were analyzed.

Results and Discussion: We obtained transmissible *null* mutant alleles for *rel*. In addition to an expected sensitivity to bacteria, *rel* mutant sand flies present higher parasites loads in their gut when infected with *L. major.* Our data show that 1) CRISPR/Cas9 technology was successfully adapted to sand flies, and 2) the sand fly immune response impacts the vector competency for *Leishmania* parasites. More generally, this study shows that CRISPR/Cas9 *in vivo* genome editing can be used to decipher the complex links between the insect immune response, the microbiota and vector competency for pathogen transmission.

Keywords: CRISPR/Cas9, sand fly, leishmania, immune response

[P3.49]

Wide distribution of *kdr* mutations in *Aedes aegypti* from Brazil

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The selection of resistance to insecticides is currently a threat to the control of *Aedes aegypti* in a worldwide scale, especially when considering pyrethroids, the most employed compounds. In Brazil, this mosquito is vector of arboviruses that have been causing alarming epidemics of dengue, chikungunya and Zika. The increase number of cases of such diseases is in part related to failure in control measures against the mosquito. Brazilian governmental campaigns have used organophosphates for three decades, which were then substituted for IGRs and pyrethroids, given the high levels of resistance registered for temephos. However, few years latter resistance to pyrethroids was detected. The main mechanism selected for this resistance is a group of mutations in the voltage gated sodium channel gene (*Na_V*), known as *kdr* (resistance to the pyrethroids knockdown effect). Here we investigated the *kdr* allelic frequency in *Ae. aegypti* populations from XXX Brazilian municipalities, simultaneously collected between 2017/18, for the alterations Val1016Ile and Phe1534Cys. For the State Capitals, we included the V410L SNP. A TaqMan SNP genotyping assay was employed for these three *kdr* sites in more than 5,500 mosquitoes. Compared to previous data, we observed that the *kdr* have rapidly spread and increased in frequency in Brazil. This might be consequence of domestic and private companies' applications of pyrethroids for urban Culicidae control. We observed the alleles Na_VS ($1016Val^{+}$+$1534Phe^{+}$), Na_VR1 ($1016Val^{+}$+$1534Cys^{kdr}$) and Na_VR2 ($1016Ile^{kdr}$+$1534Cys^{kdr}$). The 410 *kdr* mutation was present only in those individuals with mutations in both 1016 and 1534 sites. The Na_VS allele was the less frequent, being more present in the Northeastern localities. The highest frequencies were observed for Na_VR1, especially in the North and fixed in one Amazonian population. The NaVR2 was more prevalent in Central-west and South-eastern populations. We are now processing finer analyses. To our knowledge, this is the biggest nationalwide screening of a genetic mechanism for insecticide resistance and a frame picture about of how resistance to pyrethroids in Ae. aegypti is evolving in Brazil.

Keywords: vector control, insecticide resistance, arbovirus vector

[P3.50]

Triggered to death. dsRNA insecticides against the Asian Tiger mosquito, *Aedes albopictus*

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The Asian tiger mosquito, *Aedes albopictus,* is a highly invasive species that has spread rapidly in all countries of the Mediterranean basin and almost every part of the world. It is considered a major threat for public health due to its capacity to transmit many dangerous viruses, such as *Chikungunya*, *Dengue* and *Zika*. The purpose of our project is to develop an effective, species-specific insecticide for its control, through silencing of the genes that will either lead to larval death or inhibit their growth.

We analyzed available RNA sequencing data in order to identify transcripts that are exclusively expressed in larvae. We validated their expression by qRT-PCR, thus generating expression profiles throughout the life cycle of the mosquito. Our focus was on transcripts that are not conserved in other species so that they can offer multiple unique target regions for RNAi. Firstly, we *in vitro* generated dsRNA of selected loci through *in vitro* transcription and we knocked down the target genes by triggering the RNAi response through microinjection. Secondly, we cloned cDNA corresponding to each target to L4440 plasmids and transformed *E. coli* HT115 cells to create dsRNA-expressing bacterial strains which we subsequently fed mosquito larvae. The resulting phenotypes of both approaches are under investigation. In parallel, the possible role of unknown genes is being considered.

Our research aims to create an *Aedes albopictus* specific insecticide based on the RNAi technology. We have successfully identified putative gene targets and evaluated the impact of their silencing. Our next step is to transform various yeast strains with constructs expressing each dsRNA and apply our larvicidal approach under semi-field conditions. We will exploit the ability of many yeast species to act as mosquito food sources, in order to attract the larvae in the field and succeed the highest efficacy of dsRNA uptake.

Keywords: Aedes albopictus, RNAi, insecticides, Asian Tiger mosquito

[P3.51]

Structural modification of parthenin for malaria transmission-blocking

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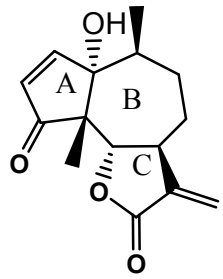
Introduction: Substantial progress has been made in the control of malaria over the past decade. However, transmission of the disease persists in many countries of sub-Saharan Africa (SSA). Concerted efforts are needed to sustain malaria control towards elimination by 2030. Amongst them are strategies that disrupt the development of the malaria parasite within its mosquito vector. Recently, we isolated parthenin [1], a sesquiterpene lactone like artemisinin, from the invasive Star weed *Parthenium hysterophorus* and found it to arrest malaria parasite development in the mosquito. Parthenin however, was found to be cytotoxic to humans. This study was designed to investigate the potency and safety profiles of synthetic derivatives of parthenin.

Methods: Parthenin was isolated from *P. hysterophorus* by soaking powdered leaves in MeOH at 25^{o}C for 24 hrs; repeated thrice, followed by concentrating under reduced pressure at 40^{o}C and purifying using various chromatographic methods. Thereafter, the structure of parthenin was modified to obtain different derivatives.

Results and discussion: We present data on the structures of the pure compounds determined by chemical analyses including Gas Chromatography (GC)-Mass spectrometry (MS), Liquid Chromatography (LC)- Mass spectrometry (MS), Liquid Chromatography Quadrupole Time-of-Flight Mass spectrometry (LC-QTOF-MS) and 1- and 2-D (13C and 1H) Nuclear magnetic resonance (NMR). Implication of the structural differences of the compounds and their possible mechanism of action is discussed. We anticipate that these compounds can act as scaffold for developing drugs that can block transmission of malaria parasite via the mosquito host.



Keywords: Malaria, Parthenin, Transmission-blocking, Star weed

[P3.52]

Overexpression of CYP6-AA2 (cytochrome P450) gene in deltamethrin resistant Anopheles stephensi

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Introduction: *Anopheles stephensi* is considered as one of the major primary malaria vector in India, Pakistan etc. In India, it contributes approximately 12% cases of malaria. Pyrethroid insecticides are currently choice of insecticides and only class of insecticide recommended for Insecticide Treated Nets (ITNs) and Indoor Residual Spraying (IRS) for the control of malaria vectors due to its rapid killing action, low mammalian toxicity and degradability in nature. Cytochrome P450 (CYPs) are important and wide class of detoxification enzymes known to play a major role in metabolic type of resistance against pyrethroid group of insecticide, by metabolizing the insecticide at a higher rate in mosquitoes. CYP6 family consist a vast range of genes like CYP6J9, CYP6-Z2, CYP6-AA3, CYP6-AA7 etc. which are known to be overexpressed and responsible for pyrethroid resistance in various insects. There is no report of pyrethroid-resistance in *An. stephensi* in India. However, in order to understand the molecular basis of insecticide resistance, we selected deltamethrin-resistant *An. stephensi* in the laboratory by providing intermittent low dose exposure to insecticide over generations. Here we report an evidence of CYP6-AA2-mediated insecticide resistance in *An. stephensi.*

Methodology: To understand the role of CYP6-AA2 gene, overexpression was monitored by synthesis of single stranded cDNA (using oligo-dt primer) from total RNA of susceptible and laboratory selected deltamethrin-resistant strain of *An. stephensi* and quantitative real-time PCR was performed by using gene specific primers.

Results & Discussion: Quantitative real-time data shows that the relative amount of CYP6AA2 transcript was significantly higher in the -resistant strain of *An. stephensi* as compared to the susceptible strain. Further study to characterize this gene in deltamethrin resistant and susceptible strain is under investigation as this gene is considered a good target and important diagnostic marker to understand the mechanism of pyrethroid resistance essential for effective integrated vector management.

Keywords: CYP6AA2, Anopheles stephensi, Deltamethrin, Pyrethroid

[P3.53]

Tick microbiome responses to the effects of urbanization

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Ticks are one of the most common vectors with a wide variety of human and animal pathogens, negatively influencing the human health and well-being. Human modification of the natural environment contributes significantly to create habitats and offers a shelter to some tick populations, in infesting not only rural, but also human residences. To follow and understand how human disturbance effects the tick microbiome, we studied the diversity and composition of bacterial communities of *Ixodes ricinus* individuals along a rural-suburban-urban gradient using 16S rRNA metagenomic approach and next generation sequencing. Our findings showed that relative abundance of bacterial communities within *I. ricinus* females was significantly higher in rural area compared to suburban and rural ones, while their Shannon diversity was significantly higher in urban area. In contrast, that of males and nymphs did not differ significantly between the studied areas. The metagenomic analyses revealed that Proteobacteria was a significantly more abundant phylum in rural females compared to suburban and urban ones. Non-metric multidimensional scaling revealed a separation in bacterial communities at every studied developmental stage of ticks between rural and urban areas indicating differences at species level. Multiple ANOVA showed that relative abundance of 23 species in nymphs, 18 species in females and 6 species in males significantly differed along the urbanization gradient. These findings suggested that changes in environmental conditions caused by urbanization resulted a shift in composition of bacterial community, particularly in female ticks. Therefore, we propose that the implementation of environmentally friendly treatments by the urban greenspace management may help to reduce the spread of tick populations and associated infectious agents in urban habitats.

Keywords: castor bean tick, disturbance, Illumina MiSeq, tick-borne disease

[P3.54]

A systematic review of the condition of visceral leishmaniasis (kala-azar) transmitted by the sand fly Phlebotomus in different parts of Iran between 2004 -2017

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Introduction:

Visceral leishmaniasis (VL), also known as kala-azar is an infectious and parasitic disease that is important in terms of health where it is caused by Leishmania donovani transmitted by the sand fly Phlebotomus. In our country, various studies have been published from different parts of the country. The aim of this study was to collect and analyze these studies in a predominantly one study A review.

Materials and Method: In this review study, we collected the published articles in the field of visceral leishmaniasis in the country using validated internal and external databases. After collecting articles, articles entering the study criteria were studied and information They were extracted, categorized and analyzed.

Results: In the last 10 papers reviewed, 19171 samples were studied in these studies. In all the articles, the DAT (Direct Agglutination Test) method was used. It has also been reported as an infectious agent in all studies of infantum species. A total of 19171 samples were studied in these ten studies. The average prevalence rate in these studies was 2.041. In these studies, the average percentage of men (52%) was higher than women (48%). The variation in the prevalence rate did not show a 14-year increase or decrease in particular.

Discussion: The results of this systematic review showed that the process of change in the past 14 years has not been incremental or decreasing, and a relatively stable trend has been reported. Northwest and Qom provinces are still endemic areas of this disease. And new reports from western provinces of the country Warnings and alarms are new and serious.

Keywords: Seroepidemiology, Visceral Leishmaniasis, Systematic Review, Iran

[P3.55]

ABC transporters in the malaria vector *Anopheles stephensi*: novel targets for the control of mosquito larvae

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Chemical insecticides still represent the backbone for the control of insect pests and vector-borne diseases, including malaria. Although, the use of high doses of chemical insecticides was crucial for malaria management, over time this led to heavy issues: the accumulation of toxic residues in the environment and the emergence of insecticide resistance in vectors. ABC transporters are one of the major components of the "defensome-machinery", involved in cellular detoxification and in insecticide resistance outbreak. Our aims were to further investigate the involvement of the ABC transporter family in insecticide detoxification in the mosquito *Anopheles stephensi*, and to inhibit the most upregulated transporter gene, in order to increase mosquito susceptibility and mortality.

The response of ABC transporter genes to insecticide treatment was investigated through permethrin induction bioassays on larvae of *An. stephensi*, followed by transcriptomic analysis. Then, inhibition tests on a selected ABC transporter gene were performed through oral administration of two different post-transcriptional silencing oligos, siRNA and antisense Vivo-Morpholino, combined with permethrin treatment.

Transcriptomic analysis revealed that different ABC-transporter genes are upregulated in their expression after permethrin treatment, suggesting their involvement in the detoxification against this insecticide. In particular, ABCG4 gene showed high upregulation. siRNA treatments targeted on the mRNA of this gene, associated with permethrin, demonstrated that ABCG4 silencing is able to increase insecticide efficacy and rise larval mortality. Finally, Vivo-MO inhibition on the same target, coherently with the results obtained using siRNA, demonstrated the potential of ABCG4 post-transcriptional knockdown as a method to increase permethrin susceptibility.

These result path the way toward the possibility to exploit ABCG4 expression inhibition to design novel approaches to control *Anopheles* mosquitoes and malaria transmission. From this perspective, Vivo-MO appear worth of further investigations, toward the development of environmental friendly mosquito larvicides.

Keywords: Vector-control, mosquito defensome, siRNA, Morpholino

[P3.56]

Yellow-g and Yellow-g2 proteins are required for egg desiccation resistance in the Asian tiger mosquito, *Aedes albopictus*

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Introduction:

Eggs from *Aedes* mosquitoes exhibit desiccation resistance that helps them to survive and spread as human disease vectors throughout the world. Several studies have suggested that eggshell/chorion melanization and/or serosal cuticle formation are important for desiccation resistance. In this study we analyzed the functional importance of two *yellow* genes, *AalY-g* and *AalY-g2*, in embryonic resistance to desiccation in eggs of the Asian tiger mosquito, *Aedes albopictus*.

Methods: Serosal cuticle formation/maturation was examined after dechorionation with NaOCl followed by TEM. dsRNA for *AalY-g* or *AalY-g2* was injected into adult females right after blood feeding. Ultrastructural defects of the eggshell were observed by TEM.

Results/Discussion: Temporal development of the serosal cuticle is not correlated with egg desiccation resistance in *Ae*. *albopictus*. Both *AalY-g* and *AalY-g2* are expressed in the ovaries from 48 h post-blood-fed females. Injection of dsRNA for either *AalY-g* or *AalY-g2* (ds*AalY-g/g2*) into adult females has no effect on the number of eggs produced. However, initial melanization is delayed by several hours with the eggshells eventually becoming a black color similar to that observed in eggs from control females. In addition, the shape of the ds*AalY-g/g2* eggs are abnormally crescent-shaped and the outermost exochorion appears to be more fragile and peeled off in places. Control eggs acquire resistance to desiccation between 18 and 24 h after oviposition (HAO). In contrast, ~70% of the 24 HAO ds*AalY-g/g2* eggs collapse when they are transfered to a low humidity environment. No electron-dense outer endochorion is evident in AalY-g- and AalY-g2-deficient eggs. These results support the hypothesis that both AalY-g and AalY-g2 are required for integrity of the exochorion as well as for rigidity, morphology and formation of the outer endochorion, a structure that apparently is critical for desiccation resistance of the *Aedes* egg.

Keywords: desiccation resistance, pigmentation/melanization, yellow-g/g2, eggshell

[P3.57]

Mechanisms of resistance to permethrin in Aedes aegypti from Chiapas Mexico

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Introduction

Pyrethroid resistance in *Aedes aegypti* has spread in Mexico, leading to difficulties in controlling disease outbreaks. To have a proper resistance management, we investigated if the metabolism to pyrethroids is also involved in a knockdown-resistant field mosquito population. The results allow diagnosing if the vector control campaigns are facing multiresistant mosquito populations, which would determine the direction for their control.

Methods

Susceptibility tests were undertaken according to CDC and kdr genotypes were determined in adult mosquitoes from Tapachula. Mosquitoes exposed during 1h to permethrin, and to permethrin and synergists (diethyl maleate [DM], piperonyl butoxide [PBO], or s,s,s, tributylphosphorotrithioate [DEM]) were homogenized, and legs removed for DNA extraction. Metabolite extraction was conducted with methanol, and derivatization handled prior to gas chromatography-tandem mass spectrometry (GCMS). Extracts were spiked with isotopically labeled internal standards for absolute quantification of the metabolites. Analyte peak areas, relative to the internal standard, were converted to ng/ml of biological sample and results associated to its phenotype and kdr genotype.

Preliminary Data

Field mosquitoes had 15% mortality to permethrin. Synergists increased permethrin toxicity raising mosquito mortalities to 50-80%. Permethrin and permethrin/DM exposed mosquitoes had a significant knockdown recovery up to 8h of incubation. Kdr allele frequencies were C1534=0.92 and I1016/L410=0.47. GCMS analysis showed 3-phenoxybenzoic acid (3-PBA) monooxigenase metabolite in permethrin and permethrin/PBO exposed mosquitoes. Esterase metabolites were not detected in GCMS.

Discussion

Permethrin resistance in this mosquito population is based on both kdr and a rapid metabolism, with esterases, cytochrome P^{450} and Gsts involved. 3-PBA metabolite was identified except in mosquitoes with esterases inhibited, indication of esterases acting in the first step of permethrin hydrolysis. 3-PBA identified in permethrin/PBO mosquitoes indicate that monooxygenases were not inhibited, as reported in another species (Brown. 1996). Using synergists combined with pyrethroids provide better control of this multiresistant mosquito population.

Keywords: kdr, metabolism, Aedes aegypti, multiresistance

[P3.58]

Investigation of vector competence of Aedes fluviatilis to Chikungunya

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The *Aedes fluviatilis* mosquito is currently transitioning from wild and rural environments to urban areas, developing in artificial containers. This fact can be a problem for public health, since this mosquito has vector competence for some pathogens, such as Dengue. It is currently unknown whether or not it can transmit urban viruses such as Chikungunya (CHIKV). Some factors increase the likelihood of susceptibility or vector competence for CHIKV as: this species is vector competent for others arboviroses. Due to these factors, we hypothesized that *Ae. fluviatilis* would present vectorial competence for CHIKV. Therefore, our aim was to test the susceptibility and vectorial competence of *Ae. fluviatilis* for CHIKV.

In order to test the hypoyhesis, *Ae. fluviatilis* from laboratory colony were orally infected with Brazilian CHIKV. We monitored virus kinetics in the head, thorax and abdomen of mosquitoes at 1, 4, 7 and 11 days post infection and a qRT-PCR was performed to determine the infection rate (IR) and dissemination rate (DR) of the virus. The IR is the proportion of mosquitoes with infected bodies (abdomen/thorax) among the total number of mosquitoes tested. DR corresponds to the proportion of mosquitoes with infected heads in relation to the body of the infected mosquitoes (abdomen/thorax).

We verified the presence of the virus in the head of the mosquitoes from the fourth day of infection, in which 80% of the mosquitoes were infected. The rate of infection and dissemination was 100% in mosquitoes with 7 days of infection. The *Ae. fluviatilis* was susceptible through transmission and infection analyzes, our next step is to verify the transmission rate (infectious saliva/infected mosquitoes) to verify the vectorial competence.

We believe that the results of susceptibility and vector competence will help guiding the control procedures of these mosquitoes, therefore avoiding the presence of one more urban vector.

Keywords: Aedes fluviatilis, chikungunya, vector competence, virus

[P3.59]

high infection rate of zika virus in mosquitoes collected from an area of active zika virus transmission of eastern thailand

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Zika virus (ZIKV), chikungunya virus (CHIKV) and dengue virus (DENV) are emerging and re-emerging arboviral diseases. These viruses are transmitted to humans through the bites of *Aedes* mosquitoes. Recently, ZIKV infection has been described as an emerging disease in Thailand and many countries, especially in tropical and sub-tropical areas. Specific drugs and vaccines against these infections are unavailable; therefore, effective disease control relies on vector control measures only. To understand the transmission cycle of these viruses and mosquito vectors, this study is designed to investigate the natural infection of ZIKV, CHIKV and DENV RNA in field-caught mosquitoes by molecular techniques. Adults and larvae of mosquitoes were collected in and around the patients' homes in the Klaeng District, Rayong Province, Thailand. CHIKV and DENV RNA were detected by Multiplex Real-time RT-PCR and ZIKV was detected by Hemi-nested RT-PCR. ZIKV RNA was detected in 8 (10.3%) samples (5 (6.4%) females and 2 (2.6%) males of *Aedes aegypti* and 1 (1.3%) female *Armigeres subalbatus*) and CHIKV RNA in 5 (6.4%) (3 (3.8%) females and 2 (2.6%) larvae of *Ae. aegypti*), while DENV RNA was not detected in any samples. The Maximum Likelihood tree of nucleotide sequences of positive samples showed that ZIKV in mosquitoes were cladded within the Asian lineage. This study was a preliminary survey of the potential vectors of ZIKV, CHIKV and DENV in an affected area. Information obtained from this study helps to understand the natural infection rates in mosquitoes with ZIKV, CHIKV and DENV and may be valuable in creating the most effective mosquito vector control strategies in the future.

Keywords: Zika, Chikungunya, Dengue, Mosquitoes

[P3.60]

Resistance to insecticides and the frequencies of *kdr* alleles in *Aedes aegypti* populations from Rio de Janeiro state, Brazil

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Brazil is experiencing outbreaks of dengue, chikungunya and Zika viruses. Eradicating *Aedes aegypti*, the vector of these arboviruses, is the best option to control them and prevent their spread. Rio de Janeiro state is a gateway of these arboviruses, due to the intense circulation of cargo and one of the main touristic destinations in Latin American. Neurotoxic insecticides remain the backbone of any control strategy, especially during epidemic bursts. However, their exaggerated use has been selecting resistant populations. The surveillance of vector populations and monitoring of their susceptibility to insecticides are imperative actions in order to keep these chemicals effective when needed. Herein we evaluated insecticides resistance in natural populations of *Ae. aegypti*, in different regions of Rio State, which accounts for a large number of cases of those diseases. Ovitraps were installed in Campos dos Goytacazes (CG), Vassouras (VS), Mangaratiba (MG), Itaboraí (IB), Itaperuna (IP) and Iguaba Grande (IG) municipalities. For adulticides, qualitative assays (WHO tube tests for PY deltamethrin 0.05% and CDC bottle tests for OP malathion 20μg/bottle) were performed while for larvicide temephos quantitative tests were realized. Qualitative tests for IGR pyriproxyfen were also performed. Meanwhile molecular assays to find the frequency of knockdown resistant (*kdr*) alleles at the voltage-gated sodium channel (Na_V) – the target of pyrethroids – were conducted. Preliminary results indicated that all *Ae. aegypti* populations are resistant to deltamethrin, but not to malathion. None of the tested six population showed resistance to pyriproxifen, while their resistant ratios to temephos were found lower than the previous 10 to 15 years, indicating the populations are probably regaining their susceptibility to temephos. The frequency of *kdr* "resistance genotypes" varied from 0.81 (for IP) to a maximum of 1.0 (for CG). These results may contribute to a better understanding of IR in *Aedes* and consequently improve its control.

Keywords: Aedes, Insecticides resistance, knockdown, IGR

[P3.61]

What's in a tick „brain"? Transcriptome of *Ixodes ricinus* synganglions

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Ticks are strict blood-feeding arthropods (Acari), which represent a potential health issue for humans and wild or domesticated animals, due to their potential to transmit disease agents. Important gaps remain in our knowledge of the tick central nervous system, for example about gene expression variations for ticks in different conditions (e.g. before versus during the blood meal). Understanding of tick synganglion activity could foster the developement of tick-specific acaricides targetting neuroreceptors, or of alternatives to chemical treatments.

We dissected synganglions of *Ixodes ricinus* adult ticks in different conditions (questing females or males, engorging females). This material was used for RNAseq on one lane of an Illumina 4000 machine. Reads were de novo assembled, mapped on a draft genome sequence, and we then focused on the transcripts with coding potential (i.e. on predicted coding sequences, n=38,909).

We obtained a tick synganglion transcriptome with a high gene completeness, which also showed a high level of specificity when compared to previously published transcriptomes for *I. ricinus*. One puzzling result was the high frequency of synganglion-specific transcripts corresponding to transposon related genes. We performed annotation and phylogenetic analyses of neuropeptides, neuropeptide receptors and neurotransmitter receptors. Notably, we achieved a near-exhaustive reconstruction of the Cys-loop Ligand-Gated Ion Channel genes (including the nAchr receptors), a first for any tick species, to our knowldege. We combined phylogenetic studies for each type of receptor with data on expression level in the different biological conditions, and on genomic localization. Populational variation was also explored, revealing highly significant polymorphisms in potential acaricidal targets.

Keywords: Transcriptomics, Phylogeny, Synganglion, Neurotransmitter receptors

[P3.62]

Gene regulatory elements and networks provide an insight into direct and indirect negative regulation by the ecdysone cascade

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Introduction: Hematophagous female *Aedes aegypti* mosquitoes transmit pathogens for diseases like Dengue, Yellow fever and Zika, while blood-feeding. Being anautogenous, these mosquitoes require a blood meal for vitellogenesis, the process of yolk formation, during maturation of eggs. Massive production of yolk protein precursors takes place in the fat bodies. Using transcriptomic data, we have previously detected that nearly half of all genes, are differentially expressed in the fat body at different time points in the post blood meal (PBM), reproductive period. Four distinct and sequential gene expression patterns were detected along with major regulators for each pattern.

Method: To decipher how complex transcription networks, govern differentially expressed genes in the fat body, during the vitellogenic period, we used bioinformatics tools to search the promoters of co- regulated gene sets. Identified putative transcription factor biding sites (TFBSs), their corresponding TFs and built putative regulatory networks. RNAi mediated depletion of selected TFs and qRT-PCR of the target genes were used for evaluation of the functionality of some of these putative TFs within the networks.

Results: We have identified 89 putative TFBSs, on the promoter regions of more than ~1400 differentially expressed genes. These putative TFBSs show clear temporal separation. JASPAR database was searched to identify the known TFBSs and their corresponding TFs. GeneMANIA webtool was used for the construction of temporally coordinated, putative regulatory networks, most likely functional during vitellogenesis. RNAi in conjunction with qRT-PCR led us to critical members of the ecdysone-receptor (EcR) mediated negative regulatory networks.

Discussion: These analyses provided an insight into the previously unknown direct and indirect negative transcriptional regulation by 20E through EcR. This study is a significant step towards the complete understanding of complex molecular mechanisms, governing the entire reproductive period, in *Ae. aegypti*, which in turn should lead to new and improved vector control strategies.

Keywords: Aedes aegypti, Dengue, Viltellogenesis, Ecdysone

[P3.63]

The Hi-C approach improved genome assemblies and revealed principles of 3D genome organization in malaria vectors

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The spatial organization of the genome plays an important role in cell function. The main principles of the 3D chromosome folding in eukaryotes have been discovered using Hi-C – a groundbreaking technology that exploits in vivo chromatin proximity information. This method can also yield dramatically improved genome assemblies. The main goals of this study were 1) to apply the Hi-C approach to improving the fragmented genome assemblies for *Anopheles* species and 2) to understand the main principles of spatial genome organization in medically important malaria vectors. We performed a Hi-C protocol on 15-hour *Anopheles* eggs to generate 10 Hi-C libraries, including two biological replicas for five *Anopheles* species, which were sequenced with Illumina 150-bp paired-end sequencing. We obtained new accurate chromosome-level genome assemblies for *Anopheles albimanus, An. atroparvus, An. coluzzii, An. merus,* and *An. stephensi.* In accordance with the Rabl-configuration model, Hi-C revealed strong centromere-centromere, telomere-telomere interactions, and long-distance interactions between chromosome arms. We identified topologically associating domains (TADs), A/B compartments, and high-frequency long-distance contacts in the *Anopheles* genomes. Functional genomics data can be now put in the chromatin organization context. Genes and transcription start sites are enriched at TAD boundaries. High-frequency long-distance chromatin contacts occur between genes and intergenic (possibly regulatory) regions. Some of these contacts are conserved across anophelines. Heterochromatin lacks typical TADs and has random interactions across the entire region. We demonstrate that Hi-C is a robust tool for visualization and discovery of chromosomal inversions. Our results provide new facts for understanding of how architectural genome folding carries into effect within the nuclear space in malaria vectors.

Keywords: Hi-C, Genome assembly, Chromatin, Mosquitoes

[P3.64]

Population genomics and chromosome analysis suggest a long history of evolutionary separation between *Cx. pipiens pipiens* and *Cx. p. molestus*

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The species from *Culex pipiens* complex are globally distributed and represent competent vectors of West Nile virus and Eastern equine encephalitis virus in both birds and mammals. *Cx. p. pipiens* and *Cx. p. molestus* currently considered as two morphological forms or subspecies that exhibit important behavioral and physiological differences. *Cx. p. pipiens* mates in open spaces, feeds on birds, and requires a blood meal for oviposition. *Cx. p. molestus*, in contrast, mates in confined spaces, feeds on mammals, can lay the eggs without a blood meal and is well adapted to human environment. However, the taxonomic status of these forms/subspecies remains unclear. Microsatellite analysis and amplified fragment length polymorphism studies identified strong genetic differences between the subspecies, however the whole-genome differentiation between them remains unstudied. We used whole-genome resequencing analysis of two laboratory colonies derived from Chicago, USA and field collections from Eurasia (Belarus and Kyrgyzstan) that represent different continents. Cytogenetic analysis was performed on Chicago colonies. Whole-genome resequencing analysis revealed strikingly different levels of genomic diversity within the two subspecies that was low in *Cx. p. molestus* genome but high and distributed along the chromosomes in *Cx. p. pipiens* genome. The inter-subspecies comparison revealed high level of genomic divergence between them (mean Fst ≈ 0.3) which was more or less uniformly distributed along the chromosomes. Phylogenomic and ADMIXTURE analyses clustered *Cx. p. pipiens* from North America and Eurasia together but *Cx. p. molestus* from both continents into two separate clades. Furthermore, mitotic chromosome analyses revealed significant length difference for chromosome 3 and distinct banding patterns in Hoechst 33342 staining between *Cx. p. pipiens* forms *Cx. p. molestus*. High level of genomic divergence and chromosome differentiation between *Cx. p. molestus* and *Cx. p. pipiens* suggest a long history of genetic isolation between the two subspecies.

Keywords: population genomics, disease vectors, cytogenetics, evolution