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FELLOWSHIP FINAL REPORT

Behavior, sensory reception and genomics in a vector insect

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REPORT INFO

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ABSTRACT

Insects vectoring human disease, like mosquitoes and kissing-bugs, endure a high risk of predation related to their life histories. Therefore, insect vectors are expected to have a finely adapted behavioral repertoire to survive in the context of their close association to vertebrate hosts. The study of molecular bases of their perception of the environment and their behavior, is relevant to understand the evolution of hematophagy as well as to promote the discovery of new targets of opportunity for developing rational control methods. Our long-lasting scientific collaboration has been dedicated to these tasks and the support of the *Le Studium* Foundation has been instrumental to further promote its development. We report here a series of studies that have been completed during the stay in the region Centre. Kissing-bugs are nocturnal insects that spend daylight hours hidden inside narrow shelters. Therefore, comprehending shelter choice, as well as the cues that trigger foraging decisions seems essential in order to predict bug distribution and activity precisely. We have focussed on *Rhodnius prolixus*, one of the two main vectors of Chagas disease in the Americas, and the way they use shelters. As an outcome of these investigations, three scientific papers have been published to report factors affecting shelter choice by bugs, the lack of a chemical marking system in bugs of this genus and the key role that host odours play in promoting bug foraging outside shelters. In parallel, we have described what seem to be diverging locomotory profiles in these bugs suggesting that foraging strategies can vary from "sitter" to "rover" individuals. As a third topic, we have developed a synthetic blend of compounds imitating the effects of the sexual pheromone attracting males to *R. prolixus* females. In a fourth topic we have uncovered what appears to be a local modulatory system present in the antennae of insects including the synthesis of transcripts for neuropeptides, GPCRs and nuclear receptors. Finally, we have revised the molecular bases of sensory processes in triatomine bugs vectors of Chagas disease in a review publication.

Keywords :
Behavior, sensory, plasticity, receptor, triatomines, vector insect

1- Introduction

Zoonoses are diseases caused by pathogens usually affecting animals, but eventually infecting humans. Insects transmitting human pathogens represent a fundamental component of the cycles of several zoonotic diseases affecting humans and their livestock. Therefore, these insect pests are currently targeted with insecticides to control pathogen transmission. Nevertheless, insecticide resistance is gradually building up in most populations under heavy xenobiotic pressure. Resistance phenomena have been reported for mosquitoes transmitting malaria, zika, chikungunya and dengue (Ranson and Lissenden 2016; Ranson et al 2010), as well as for kissing-bugs transmitting Chagas disease (Mougabure-Cueto and Picollo 2015) and sand flies vectoring leishmaniasis (WHO 2010). Alternatives to our current insecticide-based

control strategies are required in order to maintain efficacy in the control of these diseases affecting a large proportion of the human population.

Pest organisms, as well as all others, require finely tuned behavioral repertoires in order to find key resources like food and mating partners. Nevertheless, and unlike most animals, disease-vectoring insects need to avoid the deleterious defensive reactions of their hosts, that act both as sources of blood (i.e., food) and potential predators. The study of the behavior of vector insects allows understanding the mechanisms that they use to cope with the mentioned tasks, consequently becoming more capable of predicting their impacts on human health. Besides, it can help promote the development of control methods based on the behavioral manipulation of populations of these pests.

The behavioral manipulation of insect pests is a widespread concept for controlling insect species of agricultural importance (Mitchell 2012; Mafra-Neto 2013; Rodriguez-Saona et al 2012). Results based on that perspective can be attained both by inducing deleterious behaviors in the pests themselves or by recruiting natural enemies into infested areas. One of the most widespread alternatives for the behavioral manipulation of pest insects is the use of semiochemicals as trap baits or the interference of the sexual communication between females and males of a species, e.g., apple pest management (Walker et al 2017). For this task, it is important to understand the chemical signals used by pest insects, as well as the context in which they act. In its turn, the context is defined by circadian profiles, activity patterns, resting places and developmental processes.

A new avenue for the behavioral manipulation of pest insects is the application of gene-targeting techniques to pest control (Huvenne and Smagghe 2010). Post-genomic scenarios allow searching for genes that present key functions in the control of insect behavior and physiology. The search of new targets includes the functional characterization of genes coding for neuropeptides, G-protein coupled receptors (GPCRs), nuclear receptors and sensory receptors.

Our projects study all these aspects in kissing-bugs vectoring Chagas disease, as well as in mosquitoes. We report here the recent knowledge advances promoted by our collaborative initiative.

2- Experimental details

Studies dedicated to improve our understanding of shelter-related behavior are based on video-recording of bug behavior and subsequent quantitative analyses on recorded data. Most experiments have been developed in square glass arenas in which groups of insects are released and exposed to experimental conditions under which behavior needs to be characterized.

The role of bug density and light cycles on shelter use profiles

Studies evaluating the effects of bug density were performed in arenas that presented a folded piece of cardboard in a central position to allow insects to hide in a shelter. Similarly, a single shelter was offered to groups of bugs in order to study the impact of presence of a light cycle on the tendency of bugs to enter into shelters.

Do bug feces serve as chemical marks signalling active shelters in Rhodnius species?

A second series of experiments was dedicated to determine whether kissing-bugs of the genus *Rhodnius* mark their shelters with a fecal pheromone as other kissing-bugs seem to do (Lorenzo and Lazzari 1996). For this task, larger square arenas allowed presenting a dual-choice between a clean shelter or one associated with bug feces to a group of bugs. These assays were developed parallelly for four species belonging to the genus *Rhodnius*. In these experiments, feces used for tests were gathered by feeding bugs in artificial feeders and collecting their excrement simultaneously. Feces collected were used for experiments between 24 and 72 hours after deposited by bugs.

Activity patterns and bug foraging strategies

Experiments dedicated to study activity patterns were performed in a digitized 40-channel automatic actometry device developed by our group. In this system, individual bugs (up to 40 in parallel) are released in small arenas and their movements recorded by a personal computer by means of infrared LEDs acting as motor detection devices. Data recorded are fed to a computer-generated matrix that allows their subsequent analysis. The actometry study reported here applied a multivariate analysis in order to characterize whether diverging activity strategies co-exist in bug populations.

Development of a synthetic sexual pheromone for the manipulation of male bug behavior

Our group has led the study of triatomine sexual pheromones along the last fifteen years. During that interval, we were able to demonstrate that female kissing-bugs emit complex blends of volatile compounds produced by their metasternal glands in order to activate and attract males. Our main model species for these studies has been *Rhodnius prolixus*, a main vector of Chagas disease in Colombia and Venezuela. Our previous work allowed us to characterize diverse behavioral responses to female odors including the activation of sheltered males, flight initiation, the expression of odor modulated anemotaxis and male aggregation around mating pairs (Pontes et al 2013; Zacharias et al 2010; Pontes and Lorenzo 2012). As well, the studies identified the compounds emitted and their proportions (Pontes et al 2008). Here, we present experimental evidence suggesting that a synthetic blend of female pheromone components can be used to attract male *R. prolixus*.

The molecular bases of the modulation of bug antennal function

In the last ten years we have dedicated a relevant part of our efforts to the characterization of the molecular bases of bug behavior. These studies were mainly based on the development of antennal and brain transcriptomes, as well as RNAi experiments targeting candidate genes to uncover their functional properties. In this section, we report two studies focussed in this area. In a first study, we present evidence for the previously unknown expression of a series of modulatory genes in the antennae of insects. These include a series of genes coding for neuropeptides, GPCRs and nuclear receptors, as well as those of enzymes mediating their synthesis and post-transcriptional processing.

Finally, we comment on our last review of the molecular bases of sensory processes in these bug vectors, highlighting their potential for the development of environmentally sustainable and rational control tools.

3- Results and discussion

The role of bug density and light cycles on shelter use profiles

A recent paper has characterized the effect on light and bug density on the patterns of shelter use of a series of triatomine species belonging to the genus *Rhodnius* (Mosquera and Lorenzo 2020). This is the first report studying the topic in this relevant group of vectors. Briefly, the experiments demonstrated that bugs of three *Rhodnius* species react with clearly different profiles when exposed to increasing bug densities. While *R. prolixus* seems to tolerate very high-density conditions according to the very high proportion of bugs found inside shelters at all densities tested, *R. robustus* seems to be more sensitive to increasing densities, always presenting a higher proportion of insects staying outside hiding places (Figure 1). Results are then interpreted according to their impact on bug exposure and detection by control agents. Besides, the same study evinced that very high proportions of *R. prolixus* and *R. neglectus* enter shelters independent of the presence of light, while a high proportion of *R. robustus* stayed outside shelters when exposed to permanent darkness (Figure 2).

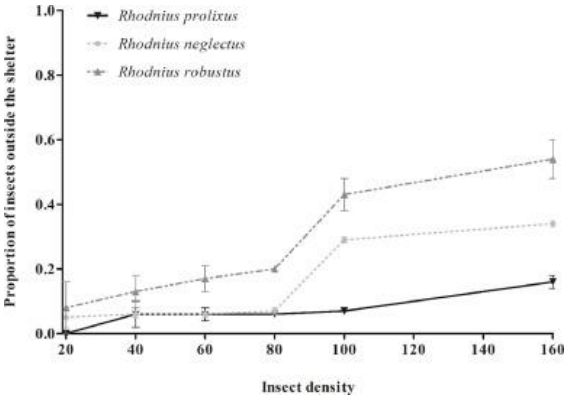

Figure 1 (original version published in *Acta Tropica*): Effect of bug density on the proportion of *Rhodnius prolixus*, *Rhodnius neglectus* and *Rhodnius robustus* 5th instar larvae not entering the central refuge.

The latter results demonstrate that the decision to enter shelters by *R. prolixus* does not depend on their negative phototaxis, as it would be expected. Furthermore, the study indicates that shelter use decisions by bugs of this species relies mostly on thigmotaxis, granting very efficient hiding performances that help them avoid detection by humans during daylight hours.

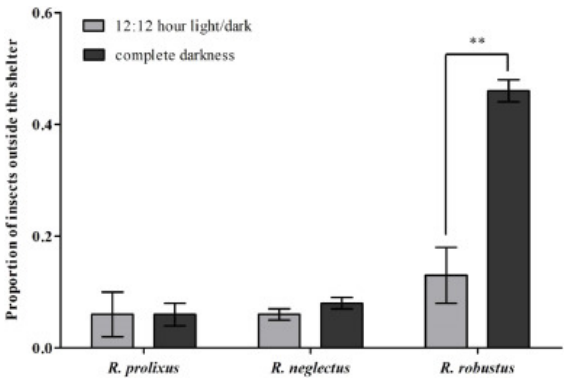

Figure 2 (original version published in *Acta Tropica*): Proportion of *Rhodnius prolixus*, *Rhodnius neglectus* and *Rhodnius robustus* 5th instar larvae not entering the central refuge under a light cycle *vs* complete darkness.

Do bug feces serve as chemical marks signalling active shelters in Rhodnius species?

A recently submitted study on this topic (in evaluation at the Journal of Chemical Ecology) has been dedicated to test whether bugs of this genus mark their hiding places with volatiles present in their feces, as suggested by previous studies (Cruz-López et al 1993; Figueiras and Lazzari 2002). Our work has tested both aggregation triggered by bug feces, as well as by a synthetic blend of fecal odors reported to promote shelter choice in triatomines of the genera *Triatoma* and *Panstrongylys*. These hypotheses were tested separately with four species: *R. prolixus*, *R. neglectus*, *R. robustus* and *R. ecuadoriensis*. In all cases, it was possible to demonstrate that odors emitted by bug feces do not act as shelter marking signals for bugs of the genus *Rhodnius*.

Activity patterns and bug foraging strategies

This actometric study allowed us to evince that foraging activity by bugs can be classified in different profiles (Marliere et al 2020). The *foraging* gene has been described in the model organisms *Drosophila melanogaster* and reported to underlie divergent foraging strategies in larval flies. Therefore, the locomotory profiles were studied with starved 5th instar larvae for which sex and the expression of the *foraging* gene orthologue *Rprofor* gene were also recorded. Our study revealed that locomotory profiles differed according to the sex of these larvae, as well as the expression of the *Rprofor* gene. As a consequence, our study reported that immature bugs already develop behavioral dimorphism based on differences in activity profiles and *Rprofor* expression, suggesting that this gene modulates locomotion in triatomines in a sex-specific manner. This, in its turn, would be the basis of diverging foraging strategies co-existing in bug populations.

Development of a synthetic sexual pheromone for the manipulation of male bug behavior

Our study, presented by Bohman and collaborators (2018), has reported a series of results demonstrating that a synthetic version of the sexual pheromone of *R. prolixus* is capable of promoting male orientation (Figure 3).

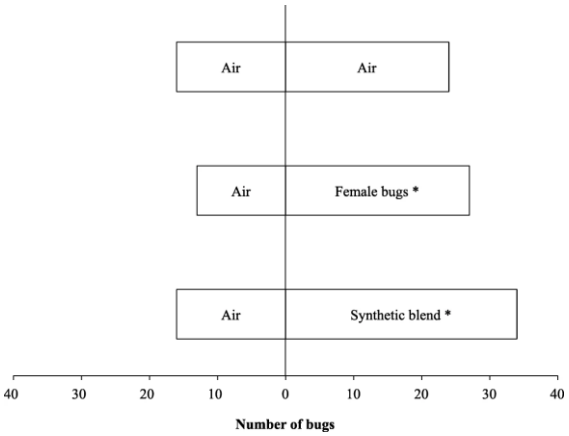

Figure 3 (original published in Parasites and Vectors): Choices by male *R. prolixus* recorded in a two-way olfactometer. Horizontal bars from above to below: First, two arms providing clean air; second, one arm providing female-emitted odour and the other clean air; and third, one arm providing synthetic odour blend and the other clean air. *P-value < 0.05 in a binomial test.

Consequently, this is the first study demonstrating that the use of a synthetic blend imitating female emitted signals can be used for attracting male triatomines. Field studies should follow in order to test whether this could have potential for application in traps or behavioral manipulation tools.

The molecular bases of the modulation of bug antennal function

The expression of neuromodulatory genes coding for neuropeptides and their receptors, as well as of other genes with modulatory roles was characterized in the antennae of larval and adult *R. prolixus*. This was performed by RNA-Sequencing (RNA-Seq) and subsequent comparison of the read abundances in libraries of 5th instar larvae, female and male adults. Our study reported a series of previously undescribed nuclear receptors, as well as takeout gene sequences. Besides, we identified the enzymes mediating the biosynthesis and processing of neuropeptides and biogenic amines in *R. prolixus*. The large set of neuromodulatory genes expressed suggests that there is a local system modulating sensory neuron physiology in insect antennae (Figure 4).

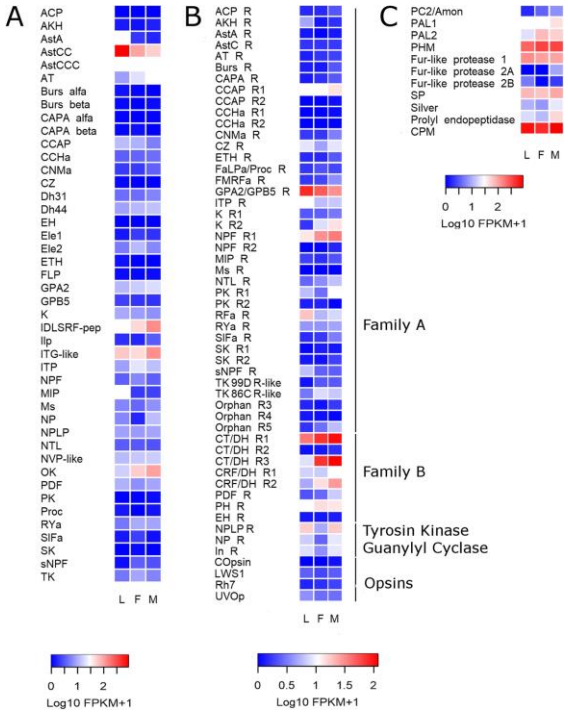

Figure 4 (original published in BMC Genomics): Heat map comparing the expression levels of (a) neuropeptide precursor genes, (b) G protein-coupled receptor genes, and (c) neuropeptide processing enzymes in the antennae of *R. prolixus* larvae (L), female (F) and male (M) adults. Expression levels (displayed as Log10 FPKM + 1) represented by means of a colour scale, in which blue/red represent lowest/highest expression.

Whether these modulatory components act on antennal sensory processes should be the focus of future studies to clarify if insects possess a peripheral system regulating sensory function and serving as an additional basis for behavioral plasticity.

4- Conclusion

An expanded body of knowledge about the behavior, sensory ecology and molecular bases of sensory plasticity of Chagas disease vectors has been presented, increasing our understanding of the biology of these relevant pest insects and opening new avenues for the development of control tools based on sustainable and rational concepts.

5- Perspectives of future collaborations with the host laboratory

We have maintained a long-lasting cooperation between our laboratories that we intend to expand. As one of the many consequences derived from the stay at IRBI, the organization of a very successful conference has promoted the development and expansion of our collaboration network. One relevant outcome is the edition of a book on the *Sensory Ecology of Disease Vectoring Arthropods* together with C. Lazzari, R. Ignell (SLU, Sweden) and S. Hill (SLU, Sweden). This initiative is under elaboration and we intend to associate its development to a Consortium proposal to be submitted to *Le Studium* for the 2021 call. The consortium will be focused on the development of the field of vector sensory ecology, as well as promote the exchanges necessary for the continuity of the networking process initiated at the Tours conference.

6- Articles published in the framework of the fellowship

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7- Acknowledgments

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