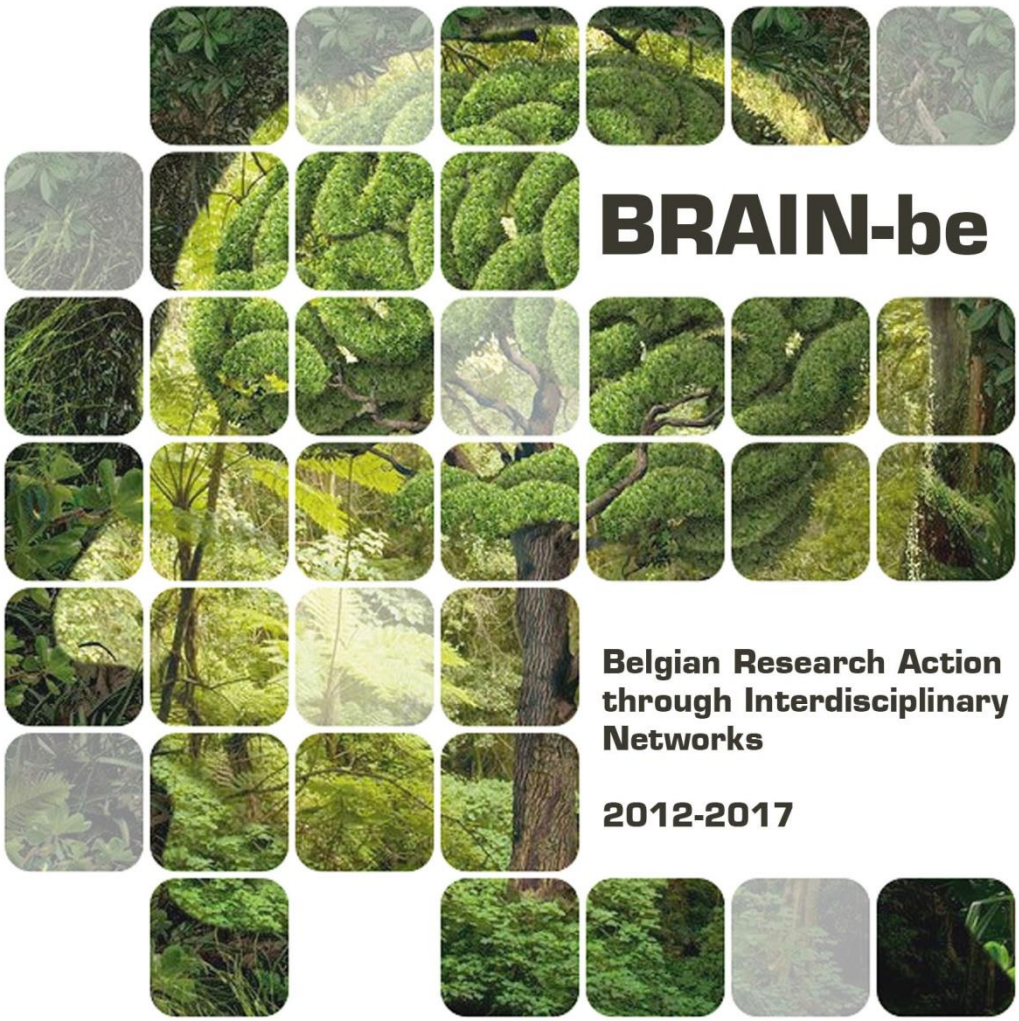

EXOTIC-BE

Experimentally Orientated genomics to Tackle Insects adaptive Challenges during bioinvasions: the laybird Harmonia axyridis as a model species

François Verheggen (Gembloux Agro-Bio Tech - ULg)

Axis 1: Ecosystems, biodiversity and evolution

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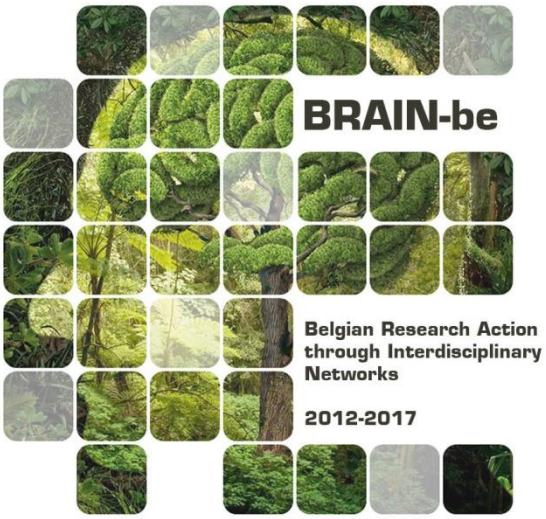

NETWORK PROJECT

EXOTIC-BE

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Contract - BR/132/A1/EXOTIC-BE

FINAL REPORT

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ABSTRACT

This project aimed to decipher the adaptive pathways underlying the global invasive success of the Asian Lady beetle *Harmonia axyridis*. It involved an interdisciplinary approach combining genomics, population genetics modeling, statistics, and experimental evolutionary biology.

The specific objective of the Belgian partner (University of Liege) consists in comparing the chemical communication of invasive and native populations of H. axyridis. Two different groups of semiochemicals have been identified from H. axyridis individuals and have been subjected to our assays: (1) cuticular hydrocarbons and (2) volatile sex pheromone constituents.

The cuticular hydrocarbons were identified for three strains (north American, European, Asiatic) and were found to be similar, with quantitative differences. Their involvement in the choice of a sexual partner has been studied in the laboratory and we found that the female mating status and the quantity of cuticular hydrocarbons does not seem to influence the choice of a sexual partner.

The volatile sex pheromone constituents were also collected. We found quantitative difference between the strains of H. axyridis. Behavioral assays were conducted and showed no ability of H. axyridis to discriminate partners originating from invasive or native populations.

All these results are subjected to peer-reviewing.

1. INTRODUCTION

The EXOTIC proposal is the result of a joined collaboration between four European partners:

- Prof Andreas Vilcinskas (Professor at Justus-Liebig Universitat Giessen, Allemagne)

- Prof Hans Vogel (Professor and AHFMR Scientist; Director BioNMR Centre, Allemagne)

- Dr Arnaud Estoup et Dr Benoit Facon (Inra Montpellier, France)

- Dr François Verheggen (Université de Liège, Belgique)

2. STATE OF THE ART AND OBJECTIVES

In order to predict the consequences of increased international trade and long distance introductions, studies have now to consider that bioinvasions are not only an ecological problem but also an evolutionary one. However, up to now, a few research programs are probing specific genetic mechanisms underlying adaptive evolution during invasions. The evolutionary forces that lead to populations with a greater propensity to invade largely remain a mystery. In light of the increasing need to control invasive species, we believe that the 'gold standard' for addressing questions about the evolutionary forces that lead to populations with a greater propensity to invade lies in the close integration of evolutionary genomics with experimental studies.

The worldwide invasion of *Harmonia axyridis* (HA) is a highly suitable context to evaluate the role of contemporary evolution in bioinvasion success. HA is native from Asia. It has long been used as a biocontrol agent against aphids but is now invasive in both North America and Europe, causing biodiversity deterioration and damage in wine industry among others. Invasion routes, as well as the nature and intensity of demographic events that have accompanied invasion (bottlenecks and admixture), have been reconstructed by the applicants. Besides applicants have been carried out several quantitative genetic studies that have demonstrated that the three types of HA populations (native, invasive and biocontrol) display phenotypic differences for several traits (generation time, cold resistance, body size and inbreeding depression) potentially linked to invasiveness. In this context, the project will aim to decipher the adaptive changes that have occurred during HA worldwide invasions for these traits using genome-wide comparisons of native, biocontrol and invasive populations. Because even based on relevant sampling strategies, blind comparisons may easily lead to potentially incomplete or incorrect adaptive hypotheses, the comparative genomics approach will be strongly orientated by targeted experimental studies, including experimental evolution towards a number of invasiveness traits. For these four traits, we will conduct experimental evolution, which in combination with genomics, will allow identifying underlying genomic regions. In addition to these, two additional traits have been suspected to underlie invasive success in HA. The first, identified in interspecific comparisons, is an exceptionally strong immune response, linked to both antimicrobial peptides and a hormone known as harmonine. The second is a very spectacular behavior of aggregation, potentially related to winter survival, and triggered by recently identified chemical compounds. For these two traits, expressed products and transcripts are known. The first step will thus be to check, using quantitative genetics designs, whether consistent differences exist between native, invasive and biocontrol populations. With all these pieces of information in hand, we will turn back to field sampled populations, and draw a genome-wide comparison of invasive, native and biocontrol populations using SNP markers.

The main expected outcomes are: (i) building of a single genomic resource framework that could be used by most scientists interested by questions related to HA (population) genomics; (ii) improving methods to detect and identify genomic region(s) under selection from populational NGS data; (iii) better understanding of the adaptive potential of populations of an emblematic invasive species for several key life-history traits; (iv) first comparison between "controlled" laboratory approaches of experimental evolution and more direct approaches focusing on populations sampled in natura; (v) delivery of the first highly documented study of the evolutionary shifts and the genetic basis of several life-history traits involving during an invasive process.

The present proposal may be seen as a pilot project on a model species (HA) for which the applicants have already good expertise (e.g., laboratory rearing, experimental manipulations) and background data (invasion history, basic population genetics data, phenotypic characterization of populations). If successful the proposed line of research may be extended to other species of agro-economical interest, handled in CBGP and farther.

3. METHODOLOGY

Key to our research program is the integration of different methods and knowledge from ecology, evolution and genomics that target several biological levels (for example genes, phenotypes, individuals and populations). This interdisciplinary approach was invaluable for detecting (and avoiding) molecular spandrels, thereby allowing more precise tests of predictions from basic evolutionary theory. The combination of these fronts of science clearly constitutes an emerging field of investigations in invasion biology and more generally in the study of the contemporary impacts of global changes. This project gainfully developped an integrative framework that combines experimental studies and population genomic in order to scrutinize the contemporary evolutionary shifts of several life-history traits that could affect the invasive process of HA.

Recent research projects in Gembloux Agro-Bio Tech (Belgium) have highlighted that HA is using pheromones, semiochemicals produced by some HA individuals attracting other individuals in the surrounding (Sloggett et al 2011; Durieux et al 2012). These pheromones comprised volatile and non volatiles molecules. One might suspect the chemical composition to have evolved in invasive individuals as an adaptive trait to the new climatic conditions of their new invaded environment.

Using analytical chemistry approaches (pheromone extraction, separation, quantification and identification) we aim at comparing the chemical composition of the pheromone of invasive HA to that of native HA and HA used in biological control. Using ethological (bioassays) and electrophysiological (electroantennography) approaches, we will evaluate the ability of the three groups to perceive and behaviourally respond to the perception of these pheromones.

4. SCIENTIFIC RESULTS AND RECOMMENDATIONS

Literature review

Chemical signals are involved in the courtship behavior of many invertebrate and vertebrate species. Lady beetles are no exception to this rule; a significant number of published reports highlight the role of cuticular chemicals involved in the reproduction of lady beetles, including gender recognition. Recent data have also demonstrated the presence of a volatile female sex pheromone that facilitates male attraction in some species. Here, we present a synthetic overview of the current knowledge about the sexual behavior of lady beetles and associated chemicals for which the function and identification have been provided. Because lady beetles are often reared as biological control agents, the chemical cues involved in their sexual behavior could be used as components in integrated management approaches against soft-bodied hemipterans. Our review provides new perspectives and potential developments for sustainable insect management mainly based on the semiochemicals involved in the sexual behavior of lady beetles. A better understanding of these chemical signals would help maximize their presence in areas where their predatory behavior is requested, as a result of the application of formulations inducing behavioral manipulation.

Results on Sex pheromone

Before the beginning of this project, no volatile sex pheromone was identified in Coccinellidae but various studies have suggested the existence of such semiochemicals. We have collected volatile chemicals released by virgin females of the multicolored Asian ladybeetle, *Harmonia axyridis* (Pallas), that were either allowed or not to feed on aphids. In the presence of aphids only, virgin females showed a stereotypical “calling behavior”, commonly associated with the emission of a sex pheromone in several Coleoptera species. These calling females were found to release a blend of volatile compounds that were found to be attractive at a distance for males but not for other females. GC-MS analyses revealed the presence of (–)-β-caryophyllene as the major constituent of the volatile blend which also comprises four other chemical components: β-elemene, methyl-eugenol, α-humulene and α-bulnesene. In a second set of experiment, the volatile emissions of the five constituents identified from the blend were daily quantified over a period of 9 days after exposure to aphids. We found that quantities of all five chemicals significantly increased over the sampled period. The results support the existence of a female sex pheromone in *H. axyridis*. These outcomes have potential in the development of more specific and efficient management methods aiming at manipulate the behavior of this invasive ladybeetle.

We also demonstrated that the females from both invasive (North America) and native (Asia) populations emit the same five pheromonal compounds such as β-Caryophyllene, β-Elemene, Methyl-Eugenol, α-Humulene, α-Bulnesene. However, each compound is emitted in larger amount by invasive females than by native ones. We could assume that such adaptation in sexual communication might be an asset for invasive settlers to communicate with conspecifics that were scattered on a wide territory at the beginning of the invasion process.

Cuticular hydrocarbons

Because of the genetic basis of cuticular hydrocarbons (CHCs) and their importance in sexual communication in lady beetles, we developed and tested two hypotheses. First, we hypothesized that the cuticular hydrocarbon profile qualitatively and quantitatively differs between females of *H. axyridis* from an invasive (Gembloux, Europe) and two native (Beijing and Tai'an, China) populations. Second, we hypothesized that males discriminate females from native and invasive populations based on CHCs profiles, and that males prefer copulating with females originating from the same population. CHCs were solvent-extracted before being quantified and identified by gas chromatography. We found no qualitative differences between females originating from native and invasive populations; however, quantitative differences were detected. Specifically, the CHC profiles of Tai'an females were more concentrated in each major saturated and unsaturated CHCs. Despite of these chemical differences, males (from native and invasive populations) equally copulated with females regardless of their origin, during multiple-choice bioassays. Our results suggest that males do not use cuticular profile to develop pre-copulatory strategy to select a mate. Post-copulatory strategies, such as the modulation of sperm quality or quantity, remain to be investigated.

Males of polyandrous species have to overcome sperm competition. They should select their mate based on the reproductive status of the female to increase their own fitness. Because the sexual behavior of lady beetles relies on semiochemicals, with cuticular hydrocarbons (CHCs) being used for mate recognition, we developed and tested two hypotheses. First, we hypothesized that the cuticular hydrocarbon profile qualitatively and quantitatively differs between virgin and mated *Harmonia axyridis* females. Second, we hypothesized that males discriminate virgin and mated females, preferring copulating with virgin females, rather than previously mated ones, to avoid sperm competition and subsequently increase their fitness. CHCs were solvent-extracted before being quantified and identified by gas chromatography. We found no qualitative differences between mated and unmated females; however, quantitative differences were detected. Specifically, the CHC profiles of mated females presented higher concentrations of alkenes, including 9-pentacosene, 9-heptacosene, and 9-hentriacontene. During dual-choice behavioral assays, males equally copulated with virgin and mated females. Our results suggest that

quantitative differences of CHC do not allow male lady beetles to identify the mating status of a potential sexual partner. We hypothesize that alternative strategies are used in this lady beetle species.

5. DISSEMINATION AND VALORISATION

This two-year project has resulted in five manuscripts that have been published, that are submitted or that are being prepared:

- Fassotte, B., Francis, F., & Verheggen, F. (2016). The scent of love: how important are semiochemicals in the sexual behavior of lady beetles? Journal of Pest Science, 89(2), 347-358. http://hdl.handle.net/2268/192121

- Fassotte, B., Fischer, C., Durieux, D., Lognay, G., Haubruge, E., Francis, F., & Verheggen, F. (2014). First Evidence of a Volatile Sex Pheromone in Lady Beetles. PLoS ONE. http://hdl.handle.net/2268/176712

- Legrand P, Vanderplanck M, Lognay G, Lorge S, Maesen P, Vilcinskas A, Vogel H, Foucaud J, Estoup A, Francis F, Facon B, Verheggen F (submitted) Do the cuticular hydrocarbon profiles of native and invasive ladybirds differ in *Harmonia axyridis*?

- Legrand P, Lognay G, Lorge S, Maesen P, Vilcinskas A, Vogel H, Foucaud J, Estoup A, Francis F, Facon B, Verheggen F (submitted) Cuticular hydrocarbon composition does not allow *Harmonia axyridis* males to identify the mating status of sexual partners.

- Legrand P, Lognay G, Lorge S, Maesen P, Vilcinskas A, Vogel H, Foucaud J, Estoup A, Francis F, Facon B, Verheggen F (submitted) First evidence of an adaptation in sexual communication between invasive and native population of the Asian harlequin ladybird.