Functional characterization of aphid salivary genes involved in host plant adaptation

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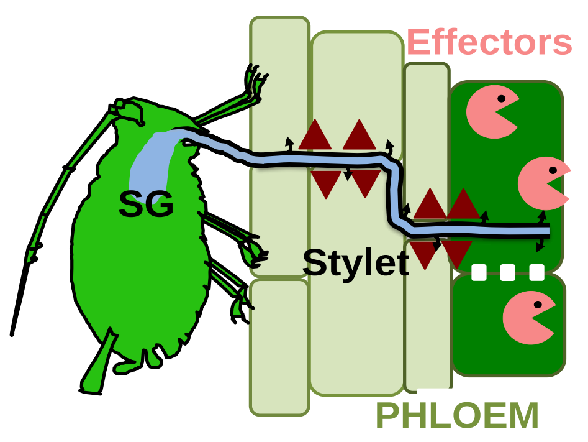
Social-economic context

Due to growing world population, there is an urgent demand to double the food production by 2050. In the meantime, we need to reduce toxic pesticide use to protect the environment. Development of the crop plants that are resistant or tolerant to pests will be one of the approaches to increase food production while minimizing pesticide use. To develop pesticide resistant crops, we need to understand how pests, such as aphids, manipulate susceptible plants, and how resistant plants defend themselves from the pest attack. The project is a fundamental research project and aims to deepen our understanding on plant-aphid interactions.

Scientific context

The pea aphid (*Acyrthosiphon pisum*), is a **sap-feeding phytophagous insect** that forms a **complex of at least 15 biotypes** each of which is specialized to one or a few species of ***Fabaceae*** (Peccoud *et al.* 2009). Establishment of aphid phloem feeding is hypothesized to be dependent on the **proteins injected into the host plant with saliva** (Hogenhout and Bos 2011, Elzinga *et al.* 2013). These proteins are thought be produced in **salivary glands (SG)**, and some are shown to **suppress or trigger plant defence** reactions like effectors of microbial pathogens (Hogenhout *et al.* 2009). Therefore, we hypothesize that pea aphid **salivary proteins are involved in host plant adaptation** and biotype formation. An **expansion of salivary gene families** through gene duplication may occur in the pea aphid due to the importance of salivary proteins in overcoming defences of a specific plant family. Some salivary gene copies may show **accelerated rates of evolution** and be under **positive selection** due to the acquisition of novel functions (neo functionalization, subfunctionalization). Moreover, some salivary genes may be polymorphic and show a **higher genetic differentiation** among biotypes (than expected under neutrality), indicating their specialization to different host plants.

APHID FEEDING

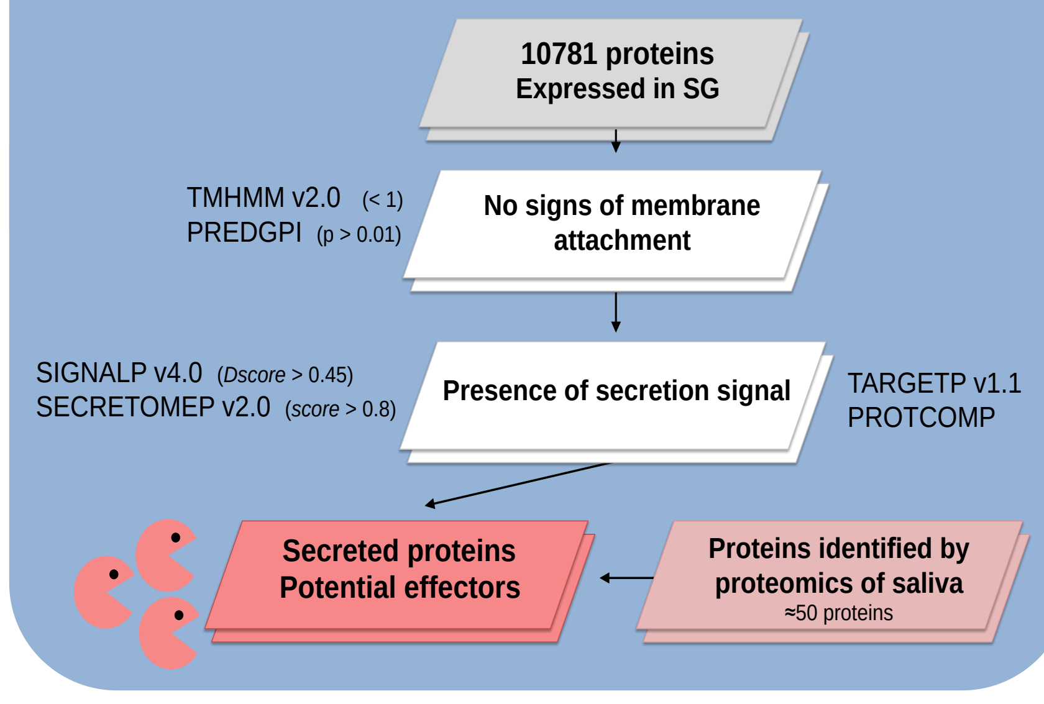


Objectives

The main objective of this study is to identify the pea aphid salivary proteins that are involved in aphid-plant interactions, in particular, host plant specialization of the biotype complex. To achieve this, we planned to identify putative secreted salivary proteins based on saliva and SG transcriptomic and proteomic datasets from *A. pisum*. Then, we will trace the evolutionary history of these potential effector genes to determine promising candidate genes for functional analysis. Finally, we will silence or overexpress the selected salivary genes and measure the aphid performances on host and non-host plants to examine the involvement of these genes in host plant adaptation.

Identification of secreted salivary proteins

1. Identify the proteins expressed in SG
2. Determine the saliva secretome



Evolutionary patterns of salivary genes

1. Large evolutionary time-scale
(Expansion of gene families ? Fast-evolving genes ? Genes under positive selection ?)

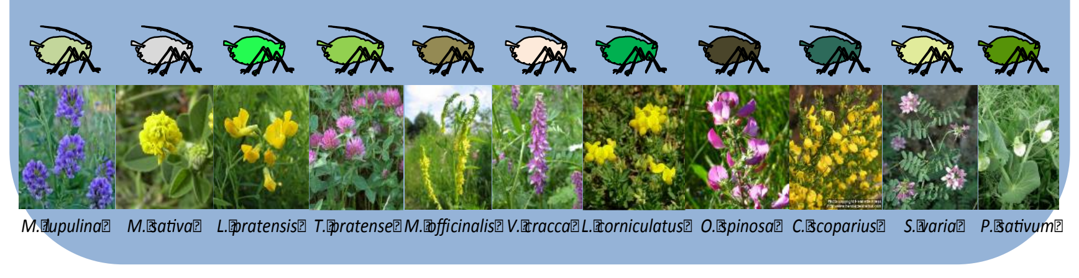
14 insect genomes
9 Hemiptera
5 others

Orthologous genes
Aphid/Hemiptera specific

Selection acting on salivary genes
dN/dS calculation

2. Short evolutionary time scale
(Genes showing a high genetic differentiation in the complex of biotypes ?)

The complex of biotypes

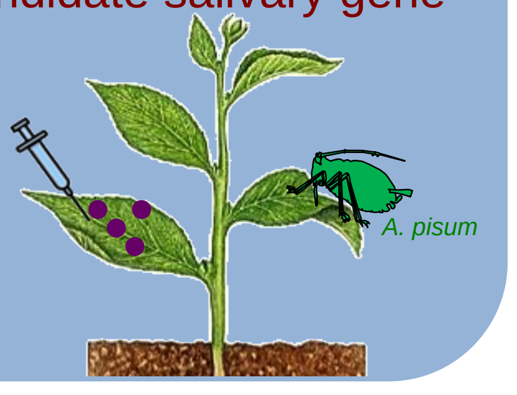


Functional characterization of salivary proteins

1. Loss of function approach
(Plant-mediated gene silencing: measure of aphid performances when a candidate salivary gene is inhibited)

2. Gain of function approach
(Gene expression *in planta*: measure of aphid performances when a candidate salivary gene is overexpressed)

Expression of dsRNA
or proteins *in planta*
by Agroinfiltration



Results

Combining published and unpublished salivary datasets, an expanded catalogue of *A. pisum* salivary proteins has been created (Carolan *et al.* in 2011 predicted 300 potential salivary effectors). The new catalogue contains 927 of the salivary proteins that are proved to be secreted with saliva or show strong evidence of secretion according to the different *in silico* predictions**.**

Perspectives

Identification and functional characterization of these salivary proteins will be the first step toward identifying the host target(s) of the saliva proteins and the mechanisms of effector recognition by non-host plants. The information regarding the targets of aphid saliva proteins will advance our knowledge on plant-sap feeding insect interactions and will help to select the crops that are not susceptible to the aphid feeding.

References : Carolan JC et al. 2011 Predicted effector molecules in the salivary secretome of the pea aphid (*Acyrthosiphon pisum*): a dual transcriptomic/proteomic approach. Journal of proteome research, 10(4); **Hogenhout SA et al.** 2009. Emerging concepts in effector biology of plant-associated organisms. Molecular plant-microbe interactions : MPMI **22**, 115–122; **Peccoud J et al.** 2009 A continuum of genetic divergence from sympatric host races to species in the pea aphid complex. PNAS 106:7495-7500; **Hogenhout SA, Bos JIB**. 2011. Effector proteins that modulate plant-insect interactions. Current Opinion in Plant Biology **14**, 422–428; **Elzinga DA, Jander G**. 2013. The role of protein effectors in plant-aphid interactions. Current Opinion in Plant Biology **16**, 451–456.