February 1, 2023
Biology International Student Selection – École Normale Supérieure

The exam in Biology is composed of three parts. During the first 30 minutes you will read and prepare your answers to the questions below (in bold). In the second part, also for 30 minutes, we will discuss your answers. In the last part of the interview, we will talk about your research and academic motivations to come and study at the ENS.

The questions below are based on a study by Xia et al. published in Cell in 2021. The topic is about evolutionary processes that allow herbivorous insects to resist plant defenses. In particular, the authors focus on the whitefly *Bemisia tabaci* which is a cosmopolitan, highly polyphagous agricultural pest that vectors several pathogenic viruses and is an excellent model to probe the molecular mechanisms involved in overcoming plant defenses.

During more than 400 million years of co-evolution with insect herbivores plants developed several different defenses. Among those, plant secondary metabolites are the most diverse and effective.

Phenolic glycosides, which comprise a sugar unit bound to a phenol aglycone, are among the most abundant plant secondary metabolites. They are toxic for the insect herbivores by affecting growth, development and behavior.

To detoxify their own secondary phenolic glycosides metabolites, plants have evolved a phenolic glucoside malonyltransferase that transforms phenolic glucoside into phenolic malonylglucoside, a nontoxic composite.

Interestingly, researchers identified in the *B. tabaci* genome the BtPMaT1 gene, a gene which carries the conserved HXXXD, DFGWG and YXGNC peptide motifs of plant BAHD acyltransferases, such as those belonging to the phenolic glycoside malonyltransferase protein family.

Sequence conservation of this gene between *B. tabaci* and several plants has been investigated and summarized in figure 1.

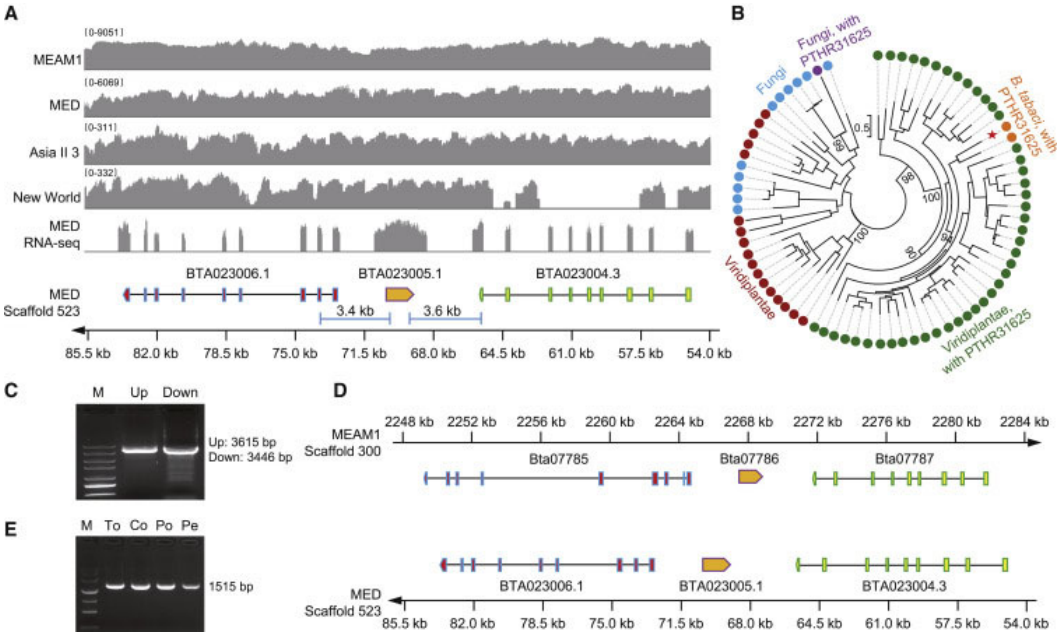

Figure 1. (A) Genomic location of BtPMaT1 (BTA023005.1) and its adjacent genes (BTA023004.3 and BTA023006.1) in *B. tabaci* MED. Genomic fragments cloned by PCR are indicated in blue. Illumina DNA-read coverage plots derived from genomic sequencing of different *B. tabaci* cryptic species and an Illumina RNA sequencing (RNA-seq) read coverage plot from adult *B. tabaci* MED are shown. The sequence depths are indicated by the numbers between brackets. (B) Maximum likelihood phylogenetic analysis of BtPMaT1. BtPMaT1 of *B. tabaci* clusters, together with BtPMaT2, within a group of plant BAHD acyltransferases containing the PTHR31625 domain. The tree is midpoint rooted, and the scale bar represents 0.5 amino acid substitutions per site. Only bootstrap values at phylogenetically important nodes are shown. BtPMaT1 is indicated by a red star. (C) Genome fragments cloned from *B. tabaci* MED. M, marker (from top to bottom: 4,000 bp, 3,500 bp, 3,000 bp, 2,500 bp, 2,000 bp, 1,500 bp, 1,000 bp); Up, BTA023004.3-BtPMaT1 genome fragment; Down, BtPMaT1-BTA023006.1 genome fragment. (D) Genome synteny of the BtPMaT1 gene and its two surrounding serine protease genes in *B. tabaci* MED and *B. tabaci* MEAM1. The reverse complement of *the B. tabaci* MED genomic fragment was taken for ease of showing the genome synteny. (E) PCR products of BtPMaT1 obtained from *B. tabaci* MED feeding on different hosts. M, marker (from top to bottom: 4,500 bp, 3,000 bp, 2,000 bp, 1,200 bp, 800 bp, 500 bp, 200 bp). Co, cotton; Pe, pepper; Po, poinsettia; To, tomato.

Question1:

Given that: 1- the BtPMaT1 location in the phylogenetic analysis; 2- the presence of two typical arthropods serine protease genes surrounding the gene in both *B. tabaci* MED and MEAM1 genomes and that 3- no orthologous BtPMaT1 gene was found in the closest arthropods at the same location or at any location of the genome; what is the likely evolution scenario that can be attributed to this gene? Describe figure 1 and explain your hypothesis.

The spatio-temporal expression profiling of BtPMaT1 was investigated in *B. tabaci*. Results are summarized in Figure 2.

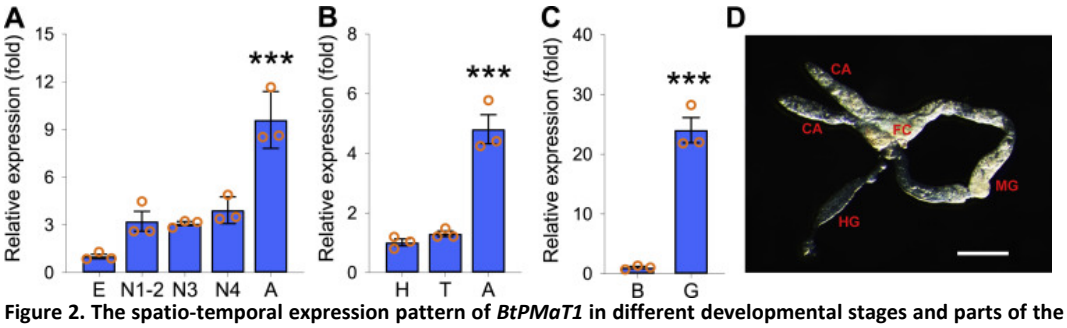

Figure 2. The spatio-temporal expression pattern of *BtPMaT1* in different developmental stages and parts of the body of *B. tabaci* MED. (A) Relative expression levels of *BtPMaT1* in eggs (E), 1st- and 2nd-instar nymphs (N1-2), 3rd-instar nymphs (N3), 4th-instar nymphs (N4) and adults (A) were determined by qPCR. (B, C) Relative expression levels of *BtPMaT1* in adult head (H), thorax (T), abdomen (A), non-gut body (B) and gut (G) were determined by qPCR. Both of the *EF1-α* and *RPL29* genes were used as internal reference genes. Values are means ± SEM, n = 3 biologically independent samples, ***p < 0.001 one-way ANOVA with Holm-Sidak's test was used for comparison. (D) The structure of the dissected gut tissues from *B. tabaci* MED adults. Abbreviations: MG, midgut; FC, filter chamber; CA, cecae; HG, hindgut. The scale bar is 100 µm.

Question 2: Describe and interpret the results of Figure 2.

Question 3: How does BtPMaT1 expression in the insect eggs support the hypothesis that you have made in question 1?

By using ultra-performance liquid chromatography/quadrupole time-of-flight mass spectrometry (UPLC-QTOF/MS), 11 representative phenolic glycosides were identified in tomato leaves and tested for their capacity to impact the performance of *B. tabaci* adults (Figure 3B). In this experiment, each compound is ingested at a dose (10uM) that is higher than the one normally ingests when feeding off the plant's phloem.

To find the function of the BtPMaT1 gene, the researchers performed dietary RNA interference (RNAi) by directly feeding its specific double-stranded RNA (dsRNA) (dsBtPMaT1) to *B.tabaci* MED adults (figure 3 A). qPCR analysis at 48h post-RNAi was examined, and *B. tabaci* survival was followed. The mortality of adults feeding on kaempferol 3-O-glucoside, kaempferol 7-O-glucoside, rhaponticin, phenyl beta-D-glucoside or phlorizin was also analyzed (Figure 3 E-I).

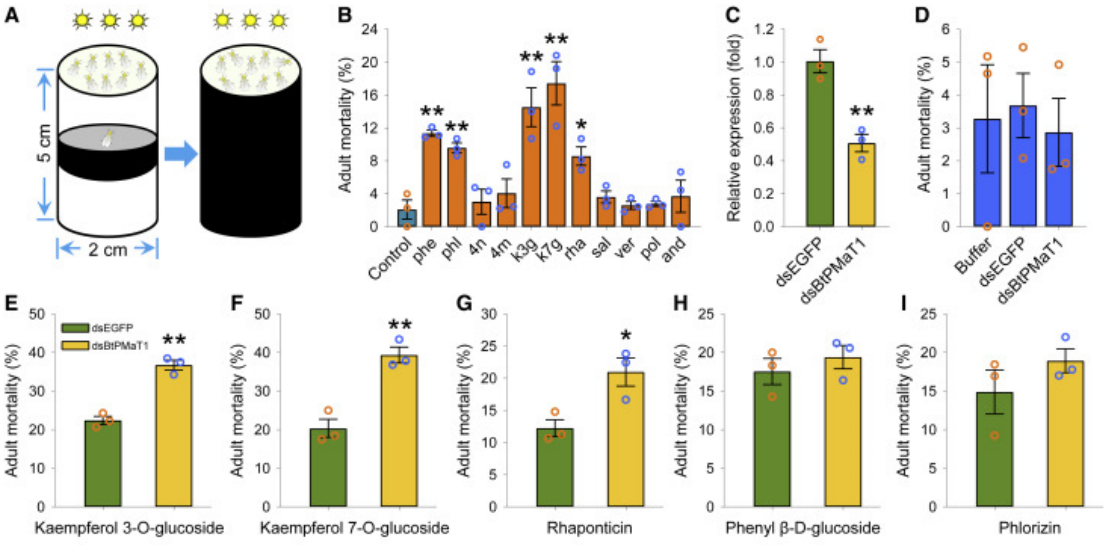

Figure 3. Effect of BtPMA1 silencing by feeding on *B. tabaci* performance. (A) A diagram of the whitefly dsRNA feeding setup. The diet containing dsRNA was placed between two Parafilm layers at the top end of a glass feeding tube (2 cm in diameter × 5 cm in length). After placing whiteflies into the tube from its bottom end, the tube was then sealed with a black cotton plug, covered with a black tube sleeve, and with the Parafilm-covered top end oriented toward a light source at ~20 cm distance. B. Effects of phenolic glycosides on the mortality of non-silenced *B. tabaci* adults recorded at 96 h. phe, phenyl β-D-glucoside; phl, phlorizin; 4n, 4-nitrophenyl β-D-glucoside; 4m, 4-methylumbelliferone glucoside; rha, rhaponticin; sal, salicin; k3g, kaempferol 3-O-glucoside; k7g, kaempferol 7-O-glucoside; ver, verbascoside; pol, polygalaxanthone III; and, androsin. (C) The transcript levels of *BtPMaT1* at 48 h post-RNAi as determined by qPCR. D) The adult mortality of B. tabaci after BtPMaT1 gene silencing (E–I) Effects of *BtPMaT1* silencing on mortality of *B. tabaci* adults feeding on different phenolic glycosides for 96 h.

Question 4: Explain the dsRNA interference assay. What other methods could have been used to inactivate gene expression?

Question 5: Explain the results in figure 3B.

Question 6: Why is it important to test the adult mortality without any treatment in the different feeding conditions in Figure 3D?

Question 7: Conclude about the role of BtPMaT1 in the physiology of *B. tabaci*.

In the next experiment, oral feeding of a representative phenolic glycoside (kaempferol 3-O-glucoside) for 48h (*i.e.* k3g) was done *B. tabaci* which then infect transgenic tomatoes (dsEFGP, for the control and dsBiPMaT1) in order to collect honeydew produced in the different experimental conditions (Figure 5A). The relative abundance of Kaempferol 3-O-glucoside (k3gm) and malonylated kaempferol

3-O-glucoside (k3gm) found in the honeydew was then analyzed (Figure 4G and 4H) and *B. tabaci* mortality recorded (Figure 4D).

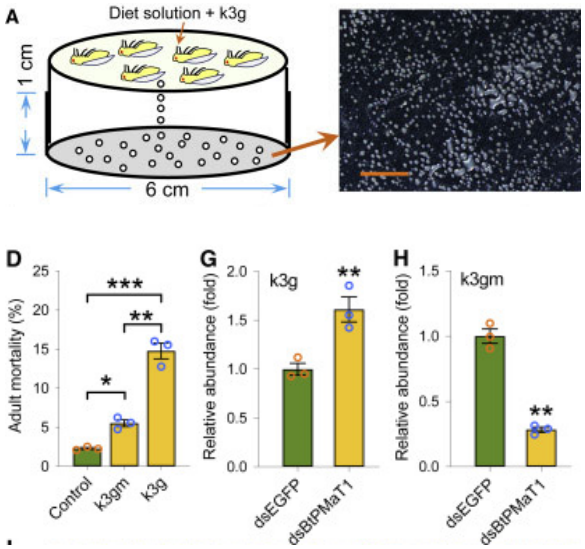

Figure 4. A. Illustration of the whitefly honeydew collection setup. The honeydew collection device consists of a vertically oriented plastic tube (6 cm in diameter × 1 cm in length) and a plastic bottom covered with tinfoil. Scale bar, 1 mm. (D) Effects of a representative phenolic glycoside k3g and its conjugated metabolite k3gm on *B. tabaci* mortality. Adult mortality of *B. tabaci* was recorded after the adults fed on diet solutions without any phenolic glycosides (control), diet solution with the k3g, or diet solution with k3gm for 96 h. G and H: Effects of *BtPMaT1* silencing on k3g (G) and k3gm (H) metabolisms of *B. tabaci*. Biologically independent samples, $^{*}p < 0.05$, $^{**}p < 0.001$, $^{***}p < 0.001$ one-way ANOVA with Holm-Sidak's test used for comparison.

Question 7. Explain the results of figure 4.

Question 8: Discuss how in general plant-herbivore co-evolution might occur by summarizing the findings shown in all figures above.