

# 1 The use of salivary $\alpha$ -amylase as an evolutionary solution to host 2 selection in parasitoids

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14

## 15 Abstract

16 Foraging parasitoids use chemical signals in host recognition and selection processes. Thereby,  
17 chemicals from the herbivore hosts play a crucial role. When different herbivores are present in the  
18 same plant or field, the perception of specific volatiles and contact compounds emitted from the host  
19 itself enable the parasitoids both to differentiate between hosts and non-hosts and to estimate the health  
20 status of its host. During the host feeding process, contact between the parasitoid and its host is very  
21 crucial, and oral secretions from the host play a key role during the first contact for such evaluation by  
22 the parasitoid. Using an integration of behavioral observations, biochemical and sensory physiological  
23 approaches we demonstrate that female parasitoids of *Cotesia flavipes* recognize their host and  
24 oviposit in reaction to an  $\alpha$ -amylase, which is present in the oral secretions of the larvae of their host,  
25 *Chilo partellus*. This activity was also mediated by a purified  $\alpha$ -amylase synthesized from *Drosophila*  
26 *melanogaster*. Using this synthesized enzyme, we further demonstrate that the conformation of the  
27 enzyme rather than its catalytic site is responsible for this activity. This enzyme is activating gustatory  
28 neurons of the terminal antennal sensilla chaetica of *C. flavipes* females.  $\alpha$ -amylases are therefore good  
29 candidates for an evolutionary solution to host selection in parasitoids, thus opening new avenues for  
30 investigations in hosts-parasitoids interactions.

31

32 **KEY WORDS:** biological control, pest insects, Lepidoptera stemborers, *Chilo partellus*, *Cotesia*  
33 *flavipes*, kairomone, host recognition by parasitoids, caterpillar oral secretion.

34

## 35 INTRODUCTION

36 One of the strategies of biological control (BC) of pest insects is based on the use of natural enemies.  
37 Among natural enemies, insect parasitoids comprise the major biological control agents (Pimentel et  
38 al., 1992; Tilman et al., 2001; Lazarovitz et al., 2007; Godfray et al., 2010), able to control insect  
39 populations in the wild (Hawkins, 1994). Among insect parasitoids, *Cotesia* is one of the most diverse  
40 genera of the subfamily Microgastrinae (Hymenoptera, Braconidae), with almost 300 species already  
41 described (Yu et al., 2016) and probably over 1,000 species world-wide, e.g. Mason (1981). Many  
42 *Cotesia* species may appear generalists but careful ecological studies may reveal a hidden complexity  
43 with an assemblage of populations having a more restricted host ranges (Kaiser et al., 2017a).

44 In sub-Saharan Africa, lepidopteran stemborers of the Crambidae, Pyralidae and Noctuidae families  
45 are economically important pests of maize and sorghum (Harris, 1990; Polaszek, 1998; Kfir et al.,  
46 2002). Due to their widespread distribution and destructive nature, stemborers have been the subject of  
47 extensive research (Calatayud et al., 2006). The most cited species are the crambid *Chilo partellus*  
48 (Swinhoe), the noctuids *Busseola fusca* (Fuller) and *Sesamia calamistis* Hampson, and the pyralid  
49 *Eldana saccharina* (Walker)(Polaszek, 1998). With exception of *C. partellus*, which was accidentally  
50 introduced from Asia into Africa before the 1930s (Kfir, 1992), they are indigenous to Africa. During  
51 the early 1990s, the International Centre of Insect Physiology and Ecology (*icipe*) renewed emphasis  
52 on BC of *C. partellus* with the introduction of *Cotesia flavipes* Cameron (Hymenoptera: Braconidae)  
53 into Kenya from Asia. The parasitoid was first released in the coastal area in 1993 (Overholt et al.,  
54 1994), where it reduced *C. partellus* densities by over 50% (Zhou et al., 2001; Jiang et al., 2006). This  
55 was to complement the action of the closely related *Cotesia sesamiae* (Cameron) (Hymenoptera:  
56 Braconidae), which is the most abundant indigenous larval parasitoid of lepidopteran stemborers in  
57 ESA. However, parasitism by *C. sesamiae* is usually below 5% though in some localities it can attain  
58 75% (Jiang et al., 2006; Kfir, 1995; Sallam et al., 1999; Songa et al., 2007).

59 The ability of parasitoids to successfully utilize cues in the two successive steps of habitat location,  
60 and discrimination between suitable and unsuitable hosts is crucial for the success of BC (Wajnberg et  
61 al., 2008; Wajnberg and Colazza, 2013). In the case of parasitoid targeting feeding host stage, the first  
62 step is often mediated by the volatile organic compounds (VOCs) resulting from the elicitation of plant  
63 defense metabolic pathways by salivary enzyme from the phytophagous host. When approaching the  
64 host, the parasitoids rely mostly on specific host-produced signals, and most of them are related to  
65 feeding activities, like fecal pellets and oral secretions (see Kaiser et al. [2017b] for a recent review).

66 Previous studies have shown that VOCs do not convey reliable information to *Cotesia flavipes* species  
67 complex, which includes *C. flavipes* and *C. sesamiae*, on the suitability of caterpillar species but they  
68 are mere indicators of the presence of herbivores (Ngi-Song and Overholt, 1997; Obonyo et al., 2008).  
69 It is only when approaching the host that reliable information on host' identity is perceived for which  
70 tactile and contact-chemoreception stimuli from the hosts play a major role in host recognition and  
71 oviposition, and it is hypothesized that protein(s) present in the host's oral secretions are involved  
72 (Obonyo et al., 2010a; 2010b; 2011).

73 In this study, an integration of behavioral observations, biochemical and sensory physiological  
74 approaches have been used to assess the nature of the active compound mediating host acceptance for  
75 oviposition, and to elucidate the mode of perception of this compound by the parasitoid, *C. flavipes*.

76

## 77 **MATERIALS AND METHODS**

### 78 **Insects**

79 *Cotesia flavipes* adults were obtained from laboratory-reared colonies established at the International  
80 Centre of Insect Physiology and Ecology (*icipe*), Nairobi, Kenya. The colony originated from  
81 individuals collected in the field in the coastal region of Kenya in 1998. Field collected *C. flavipes*  
82 were added twice a year to regenerate the colony. The parasitoid was reared on *C. partellus* larvae  
83 according to the method described by Overholt et al. (1994). Parasitoid cocoons were kept in a Perspex  
84 cage (30 cm x 30 cm x 30 cm) until emergence. Adult parasitoids were fed on a 20% honey/water  
85 solution presented. They were then put under artificial light and left for 24 h to mate. In all the  
86 behavioral bioassays, only 1-day-old naïve, mated females were used. Experimental conditions were  
87 maintained at  $25 \pm 2$  °C, 50–80% relative humidity (RH), and a 12:12 h (L:D) photoperiod (Overholt  
88 et al., 1994).

89 The host *C. partellus* originated from maize grown in the coastal region of Kenya. The larvae were  
90 reared on the artificial diets described by Ochieng et al. (1985). Thrice a year feral stemborer larvae  
91 from the coastal region were added to rejuvenate the colonies.

92

### 93 **Collection of oral secretions from *Chilo partellus* larvae**

94 It is known that acceptance of *C. partellus* host larvae for oviposition by *C. flavipes* is enhanced when  
95 the host larvae were fed on maize stems for 24h prior exposure to parasitism (Mohyuddin *et al.* 1981;  
96 Inayatullah, 1983; Van Leerdam *et al.*, 1985; Potting *et al.*, 1993; Overholt *et al.* 1994). Therefore, to

97 isolate the semiochemicals of the oral secretions of *C. partellus* that can be involved in host acceptance  
98 of *C. flavipes*, we used larvae previously fed for 24h on their original host plant (maize stems) and also,  
99 for comparisons, on stems of an alternative host, *Penisetum purpureum* Schumach. (Poaceae),  
100 surrounding frequently maize farms in Kenya. We compared also the behaviour of *C. flavipes* towards  
101 these two types of oral secretions with oral secretions of larvae fed on artificial diet of Ochieng *et al.*  
102 (1985). In addition, to verify if these semiochemicals are synthesized when the host are feeding. We  
103 compared also the oral secretions from starved larvae for 48h. For each type of oral secretions  
104 collection, a single larva held by a soft forceps was squeezed behind the head and capillary tube was  
105 used to collect oral secretions and placed directly on ice. The process was repeated for several larvae.  
106 The volume of oral secretion was estimated by weighting. All samples were preserved at -80°C before  
107 use. As evoked at the introduction, in a previous study it was hypothesized that the semiochemicals  
108 from oral secretions involved in host recognition by *C. flavipes* might include enzymes or thermo-  
109 labile proteins (Obonyo *et al.* 2010b). Therefore, we compared also oral secretions from larvae fed on  
110 maize stems but previously treated by proteinase K (Sigma product P6556) in order to destroy the  
111 proteins present in the oral secretions. In summary, the following types of oral secretions were  
112 compared:

- 113 - from starved larvae;
- 114 - from larvae fed on maize stems;
- 115 - from larvae fed on *P. purpureum* stems;
- 116 - from larvae fed on artificial diet;
- 117 - from larvae fed on maize stems followed by proteinase K digestion.

118

### 119 **Behavioral bioassays**

120 In previous studies, we demonstrate that the parasitic wasps exhibit antennation (=use of antennae to  
121 prospect by drumming the body of the host) followed by at least one stinging attempt (one tentative of  
122 insertion of their ovipositors in the host) to accept a caterpillar as a host for oviposition (Obonyo *et al.*,  
123 2010a; 2010b). Therefore, in this study we used these behavioral steps to evidence host acceptance by  
124 *C. flavipes*. To test the behavioural activities triggered by different extracts (i.e. type of oral secretions,  
125 electrophoretic bands and known proteins, see previous and next sections), they were placed on small  
126 cotton wool presented to female wasps. A small piece of cotton wool was rolled into spherical shape  
127 (around 2 mm in diameter) and placed at the centre of a Petri dish of 8 cm diameter without the Petri  
128 dish cover. About 0.5 to 1 µl of the extract to be tested was deposited on the cotton wool ball while  
129 ensuring that the cotton wool was kept moist but not wet. A single female wasp was introduced near

130 the cotton wool and both were covered with a transparent circular Perplex lid (3 cm diameter, 1 cm  
131 height) to prevent the parasitoid from flying off and to allow the observations.

132 The behaviour of the wasp in the Petri dish was then monitored for a maximum of 120 s. For each  
133 wasp, both the antennation and stinging attempt were recorded. The percentage of positive response  
134 (i.e. antennation + stinging) was calculated from 10, 20 or 30 wasps tested per electrophoretic bands,  
135 per type of oral secretions or per identified proteins (see previous and next sections), respectively. The  
136 wasp, the cotton wool ball with tested extract and the arena were replaced each time between each  
137 observation.

138 All behavioural experiments were carried out in a room with temperature of  $26 \pm 1$  °C between 10h00  
139 to 14h00 with a constant source of light to maintain an optimal temperature for the behavioural  
140 activities of the female wasps.

141

#### 142 **Electrophoresis and isolation of proteins from polyacrylamide gel**

143 The oral secretions from *C. partellus* were first centrifuged at a maximum speed of 14,000 ×g for 5  
144 minutes in order to remove the undetected debris (frass and undigested food materials). This was  
145 followed by desalting and concentrating the samples using Amicon® Ultra-0.5 centrifugal filter  
146 devices (Merck Millipore). The samples were quantified before electrophoresis using the Pierce BCA  
147 protein assay Kit (Thermo Scientific No. 23227) based on bicinchoninic acid (Smith et al., 1985). All  
148 the quantification measurements were carried out using Eppendorf-Biospectrometer fluorescence  
149 machine (SN 667).

150 Electrophoresis was conducted under non-denaturing conditions (native PAGE electrophoresis,  
151 Ornstein-Davis discontinuous buffer system) according to the method described by Chrambach and  
152 Jovin (1983) and Niepmann and Zheng (2006). The gels were cast in two sections using the Bio Rad  
153 Mini-PROTEAN® Electrophoresis System and Hoefer™ Mini Vertical Electrophoresis Systems  
154 (Fisher Sci.com). A stacking gel (4%T, 2.7%C, 0.125M Tris-Cl pH 6.8) was cast on top of a resolving  
155 gel of (7.5%, T4.4%C, 0.125M Tris-Cl pH 6.8). Electrophoresis was conducted (running buffer:  
156 0.025M Tris, 0.192M glycine pH 8.3) immediately after loading the samples at a constant voltage of  
157 150V and current of 25mA for 1-2hr in a cold room. At the end of the run, gels were immediately  
158 removed and stained for 30 min in a staining solution consisting of 0.2 % Coomassie Brilliant Blue  
159 R250. The gels were then destained with a solution of methanol, glacial acetic acid and water at the  
160 ratio of 4:1:5. The stained proteins were compared with a molecular mass standard (Sigma Aldrich)

161 containing albumin from bovine serum (Sigma A8654, 132 kDa), urease from jack bean (Sigma  
162 U7752, 272 and 545 kDa),  $\alpha$  lactalbumin from bovine milk (Sigma L4385, 14.2 kDa) and albumin  
163 from chicken egg white (Sigma A8529, 45 kDa).

164 For the isolation of electrophoretic bands, the protein bands were manually excised from the gel before  
165 staining process following the method of Kurien and Scofield (2012) with some modifications. The  
166 excised gel fragments containing the protein of interest were frozen overnight at  $-80^{\circ}\text{C}$ . Each frozen  
167 gel fragment was ground using a mortar into fine powder under liquid nitrogen and the resulting gel  
168 powder transferred to the upper chamber of the Costar® column (centrifuge tube filters, Costar lot No.  
169 22304012 Corning incorporated, NY 14831-USA). The protein trapped in the gel powder was eluted  
170 using native elution buffer 0.25M Tris HCl buffer pH 6.8, or normal saline depending on the  
171 subsequent application. After 10 min of centrifugation at  $13000 \times g$ , 300 to 350  $\mu\text{l}$  of the filtrate was  
172 recovered and stored for further concentration and desalting. A second elution was performed with  
173 fresh elution buffer and a filtrate of approximately 250-300  $\mu\text{l}$  was collected and combined with the  
174 previous one. Each protein eluted was concentrated 25-30  $\times$  folds using a Amicon centrifugal device  
175 equipped with 30K MWCO omega membrane. The concentrated protein eluents were assayed for  
176 protein content with the aforementioned Pierce BCA protein assay Kit. For each protein eluent, the  
177 purity and elution efficiency were checked by native PAGE electrophoresis. Proteins in the gel were  
178 Coomassie-stained as above. All the 7 major bands revealed in the oral secretion of *C. partellus* fed on  
179 maize (see Fig. 1) were separated and purified as described above for use in behavioural assays (see  
180 previous section).

181

## 182 **Protein identification**

183 The gel purified protein eluent inducing parasitoids' host recognition and oviposition were identified  
184 using LC-MS/MS. The protein eluent were first denatured in Laemmli buffer and then concentrated  
185 using a short electrophoretic migration, which also allowed removing any contaminants that could  
186 interfere with the trypsin digestion. Electrophoretic bands were excised and the gel pieces were washed  
187 in successive baths consisting of 50mM ammonium bicarbonate and acetonitrile. Proteins were then  
188 reduced by 10 mM of 1.4 dithiothreitol (DTT) and alkylated with 55mM of iodoacetamide to block the  
189 sulfide bonds of cysteines. After rinsing to remove residues of DTT and iodoacetamide, proteins were  
190 hydrolyzed by the addition of 0.125 $\mu\text{g}$  trypsin for 7 hours. After hydrolysis, the resulting peptides  
191 were extracted from the gel pieces with 50% acetonitrile acidified with 0.5% of trifluoroacetic acid  
192 (TFA). After complete speed vac drying, peptides were resuspended in a solution of 2% acetonitrile,



193 0.05% formic acid and 0.05% trifluoroacetic acid. Peptide mixes were then analyzed by LC-MS/MS  
194 using a nanoRSCL (thermoFinnigan) coupled with a LTQ Orbitrap Discovery (Thermo). The samples  
195 were loaded on a PepMap100C18 trap column for 5min with 2% acetonitrile (ACN), 0.08% TFA qsp  
196 H<sub>2</sub>O. Two buffers systems were used to elute the peptides: 2%ACN and 0.1% formic acid in water  
197 (buffer A); 98% ACN and 0.1%formic acid in water (buffer B). Peptide separation was performed  
198 using a linear gradient from 4% to 38 % of buffer B in 15min. The nanoHPLC was connected to the  
199 mass spectrometer using a nano electrospray interface (non-coated capillary probe 10 $\mu$  I.d. New  
200 objective). Peptides ions were analyzed using Thermo Xcalibur (version 2.0.7) using the following  
201 data dependant steps: (1) full MS scan with a 300 to 1400 m/z range in the Orbitrap with a resolution  
202 of 15,000; (2) fragmentation by CID in the linear trap with a normalized energy at 35%. Step 2 was  
203 repeated for the three most intense ions with a minimum intensity of 500. Dynamic exclusion was set  
204 to 30 seconds.

205 Raw files were converted to the mzxml format using msconvert (3.0.9576  
206 <http://proteowizard.sourceforge.net/tools.shtml>). Database search was performed using X!tandem  
207 JACKHAMMER (Craig and Beavis, 2004). Tolerance was set to 10 ppm for precursor ions and 0.5 Th  
208 for fragment ions. Cys-carboxyamidomethylation was set to static modification. Methionine  
209 oxydation, Nter acetylation of proteins, glutamine Nter deamidation and glutamic acid Nter water loss  
210 were set to variable modifications. Three databases were used: the *Spodoptera frugiperda* (Smith) EST  
211 database (<http://www.ncbi.nlm.nih.gov/nucest> version 2015, translated in the six reading frames and  
212 filtered to a minimum of 80 amino acids; 392,538 entries); the *Zea mays* database (from maizegdb,  
213 version v5a; 136,770 entries) and a standard contaminant database (55 entries). Identified peptides  
214 were filtered using X!tandemPipeline v3.3.4 (Langella et al., 2016) with the following criteria: peptide  
215 E-value less than 0.03, minimum 2 peptides per protein, protein E-value less than 10<sup>-4</sup>. Unassigned  
216 spectra were subjected to *de novo* identification using denovopipeline v1.5.1  
217 (<http://pappso.inra.fr/bioinfo/denovopipeline/>), that allows the selection of unassigned spectra of good  
218 quality and their submission to pepnovo (v2010117, Frank 2005). Spectrum quality score was set to  
219 0.2 and pepnovo score to 70. *De novo* sequences were then aligned to the same databases as for  
220 X!Tandem search using Fasts.v36.06 (Mackey et al., 2002). Proteins with a homology score less than  
221 10<sup>-4</sup> were validated. The biological and analytical reproducibility were addressed by a quantitative  
222 western blot (see next section).

223 Identified EST sequences obtained from digested peptides were submitted to a BLAST procedure  
224 (BLASTX, NCBI). The resulting protein was characterized by the name, the source and the molecular  
225 weight and a E-value/log E-value coverage. In order to calculate the coverage per cent of a peptide, the

226 EST sequence was translated into a protein sequence using the ExPasy Translate tool ([http://](http://www.expasy.org/tools/dna.html)  
227 [www.expasy.org/tools/dna.html](http://www.expasy.org/tools/dna.html)).

228

229

### 230 **Western blot analysis of the protein eluent inducing parasitoid oviposition**

231 In order to confirm that the proteins purified and identified were indeed  $\alpha$ -amylases, we performed a  
232 western blot using an antibody specific to *Drosophila melanogaster* Meigen  $\alpha$ -amylase. Ten  
233 microliters of each heat denatured protein sample (of about 500 ng/ $\mu$ l) were loaded on a NuPAGE 4-  
234 12% Bis-Tris Gel (Invitrogen) and electrophoresis conducted for one hour at 200 volt in MOPS buffer.  
235 The proteins were then transferred to an iBlot Gel Transfer Nitrocellulose membrane (Invitrogen)  
236 using the iBlot Gel Transfer Device (Invitrogen). The membrane was washed in 1X PBS for 20  
237 minutes, after which it was incubated for 90 minutes in a milk solution (1X PBS, 0.1% Tween, 5%  
238 milk) in order to saturate the membrane with proteins. The membrane was then incubated with the  
239 primary anti *Drosophila melanogaster*  $\alpha$ -amylase antibody, kind gift from Dr B. Lemaitre (Chng et al.,  
240 2014), 1000-fold diluted in a solution of 1X PBS, 0.1% Tween, 1% milk) for several hours. After this  
241 step, the membrane was washed six times in 1X PBS, 0.1% Tween before incubating with the  
242 secondary antibody (Anti guinea pig IgG Peroxidase, Sigma A7289), 1000-fold diluted in a solution of  
243 1X PBS, 0.1% Tween, 1% milk, for one hour. The membrane was then washed 3 times in 1X PBS,  
244 0.1% Tween. The peroxidase activity was detected with Amersham ECL Prime Western Blotting  
245 Detection Reagent (GE Healthcare) and recorded on an Odyssey FC imager.

246

### 247 **Sources of different $\alpha$ -amylases assayed**

248 To confirm the involvement of  $\alpha$ -amylases in host acceptance and oviposition by *C. flavipes*, we used  
249 well-purified and well-identified  $\alpha$ -amylases from different organisms available in the commerce or in  
250 our lab. at Gif-sur-Yvette: the micro-organism, *Aspergillus oryzae* (Ahlburg) E. Cohn, the insects,  
251 *Drosophila melanogaster* and *Chilo suppressalis* (Walker); and the pig as a mammal (porcine  
252 pancreas).  $\alpha$ -amylases from *A. oryzae* and porcine pancreas were obtained from Sigma No A9857 and  
253 A3176, respectively. The  $\alpha$ -amylase from *Drosophila melanogaster* was produced in the yeast *Pichia*  
254 *pastoris* (Guillierm) Phaff, as described in Commin et al. (2013). The  $\alpha$ -amylase of *C. suppressalis*  
255 was also produced in *P. pastoris*: the coding sequence of the *C. suppressalis* amylase gene 108827 was  
256 synthesized (Eurofins MWG), with replacement of the signal peptide by the one of *D. melanogaster*  
257 amylase (suppl. Fig. S1). We assayed an amylase from *C. suppressalis*, because its genome is available,  
258 contrary to *C. partellus*. In addition, to check if the behavioural activities of *C. flavipes* triggered by  $\alpha$ -



259 amylase (see results) was due to the structural conformation and/or the catalytic activity. We  
260 synthesized an inactive  $\alpha$ -amylase with no change in its structural conformation. An inactivated  $\alpha$ -  
261 amylase of *D. melanogaster* was obtained by a single replacement of the crucial catalytic residue  
262 Asp186 by an asparagine, which does not change the structural conformation (Aghajari et al., 2002). A  
263 colorimetric activity test (Infinity Amylase Reagent, Thermo Fisher) was used to confirm that this  $\alpha$ -  
264 amylase of *D. melanogaster* had no catalytic activity.

265

### 266 **Electrophysiological responses from wasp antennal sensilla towards $\alpha$ -amylases**

267 Similarly to Iacovone et al. (2016), electrical activity was recorded from antennal sensilla chaetica of  
268 the female wasp in response to the protein extract and reference compounds using the tip-recording  
269 technique (Hodgson et al., 1955). Female wasps (1–3 days old) were secured to a platform using thin  
270 strips of adhesive tape. The insect was grounded via a silver wire, bridged to the insect body by a drop  
271 of electrolyte gel (Redux® Gel, Parker laboratories, Inc. Fairfield, NJ). Individual sensilla chaetica  
272 were contacted at the tip with a glass electrode containing the taste solution and an electrolyte  
273 (tricholine citrate 30 mM) which ensures a good electrical contact as well as inhibits the gustatory  
274 neuron to water and elicits not more than 8 spikes/s (Fig. 5). In *Drosophila*, tricholine citrate ensures a  
275 good electrical contact and inhibits the water cell (Wieczorek and Wolff, 1989). Taste responses were  
276 recorded for 2 s and were performed under a microscope (Z16 Apo, Leica France). Electrodes  
277 (borosilicate glass capillaries, 1.0 mm O. D. x 0.78 mm I. D., Harvard Apparatus) with a tip diameter  
278 of approximately 10  $\mu$ m were pulled using a laser electrode puller (Model P-2000, Sutter Instrument  
279 Co, USA).

280 The recording electrode was connected to a preamplifier (gain = x10; TastePROBE DTP-02, Syntech,  
281 Hilversum, The Netherlands) (Marion-Poll and van der Pers, 1996), and the electric signals were  
282 further amplified and filtered by a second amplifier (Cyber-Amp 320, Axon Instrument, Inc., gain =  
283 x100, eight-order Bessel pass-band filter = 10–2800 Hz). These signals were digitized (DT9818, Data  
284 Translation; sampling rate = 10 kHz, 16 bits), stored on computer, and analysed using dbWave  
285 (Marion-Poll, 1996). Spikes were detected and analysed using software interactive procedures of  
286 dbWave. We evaluated the action potential frequency by counting all spikes occurring during the first  
287 second of recording.

288 The responses to the following stimulants were recorded extracellularly and compared using 30 mM  
289 tricholine citrate (all compound tested were suspended into this solution to inhibit the gustatory neuron  
290 to water) as control:

- 291 - oral secretion of *C. partellus* as a control;
- 292 - purified  $\alpha$ -amylase from *D. melanogaster* and *C. suppressalis* at 300 ng/ $\mu$ l (the concentration of the
- 293 band n°4 that was inducing oviposition in *C. flavipes*, see results);
- 294 - BSA at 300 ng/ $\mu$ l (as a standard protein of 55-60 kD, molecular weight close to  $\alpha$ -amylase).

295

## 296 **Statistical analysis**

297 The Marascuilo's procedure was used to separate the percentages of wasps that exhibited positive  
298 responses (i.e. antennation + stinging attempts) (Marascuilo, 1966). For bioassays with known proteins,  
299 the percentage of positive response was calculated from a group of 5 wasps replicated 6 times (i.e.  
300 n=6). A non-parametric Kruskal-Wallis test was applied with type of proteins as factor. ANOVA was  
301 not used because none of the data were normally distributed and had homoscedastic variance.  
302 Following Kruskal-Wallis test, a pairwise Wilcoxon's rank sum test was conducted with false  
303 discovery rate (FDR) correction for multiple testing. Comparisons among sensilla chaetica responses  
304 towards oral secretions of *Chilo partellus* and different proteins were conducted using one-way  
305 analysis of variance (ANOVA) with the Tukey's contrast test for multiple comparisons between means.  
306 Before running this ANOVA, the homogeneity of variance and data normality were examined by F -  
307 test and Kolmogorov-Smirnov methods. These statistical analyses were done in R version 3.3.1 (2016).

308

## 309 **RESULTS**

310 The oral secretions of *C. partellus* previously fed on maize stems induced significant antennation and  
311 stinging attempt (Table 1). The *C. partellus* oral secretions from larvae previously fed on *P.*  
312 *purpureum* triggered as many responses as the one from maize-fed host larvae. Comparatively, oral  
313 secretions of larvae fed on artificial diet did not elicit any behavioral activity. In addition, the oral  
314 secretions from larvae starved for 48h did not elicit any behavioral response as well as when the oral  
315 secretions from larvae fed on maize stems were treated with proteinase K.

316 The electrophoretic analyses of the active oral secretions revealed the presence of more intense  
317 electrophoretic bands (i.e. higher quantities of proteins) than of the inactive oral secretions, confirming  
318 the involvement of protein(s) in triggering antennation and stinging attempt (Fig. 1A).

319 The oral secretion of larvae fed on maize stems showed seven major electrophoretic bands in a one-  
320 dimension gel electrophoresis under non-denaturing conditions (Fig. 1A). Each major band was  
321 manually excised from the gel, extracted (Fig. 1B) and tested for further behavioral responses as

322 shown in Table 2. Out of these seven protein bands, only two bands elicited activity, particularly band  
323 no 4 ( $\approx 50$  kDa) which triggered the highest response, i.e 90% of *C. flavipes* exhibited antennation and  
324 stinging attempt (Table 2). It was thus subjected to further analysis and identification.

325 In order to identify the active protein band that induced the highest behavioral response, proteins from  
326 band No 4 were digested and the resulting peptide mixture was analyzed by liquid chromatography-  
327 mass spectrometry. Database search allowed the identification of two distinct maize proteins with 5  
328 and 2 peptide sequences, respectively, while *de novo* sequencing allowed the identification of 22  
329 peptides that matched to accession gi|295290041|gb|FP379314.1|FP379314| of the *S. frugiperda*  
330 database of mid gut cDNA sequences (Supplementary Table 1). The protein sequence blasted  
331 significantly with  $\alpha$ -amylase superfamilies (Fig. 2). The confirmation of  $\alpha$ -amylase assignment of the  
332 electrophoretic band no 4 was done by western blot analysis (Fig. 3). The anti- $\alpha$ -amylase of *D.*  
333 *melanogaster* linked mostly with the band no 4 ( $\approx 50$  kDa) of the oral secretion of *C. partellus* and  
334 with that extracted from the gel.

335 The activity elicited by different  $\alpha$ -amylases from different origin, including  $\alpha$ -amylase of *D.*  
336 *melanogaster*, confirmed the involvement of this enzyme in *C. flavipes* antennation and stinging  
337 attempt (Table 3). In contrast, the use of a different protein such as BSA did not induce any behavioral  
338 response in the wasp. The  $\alpha$ -amylases from insects, i.e. *D. melanogaster* and *C. suppressalis*, induced  
339 the highest behavioral responses in *C. flavipes* antennation and stinging attempt although not  
340 significantly different to the responses induced by *A. oryzae*  $\alpha$ -amylases (Table 3). To check if the  
341 behavioral activity of *C. flavipes* triggered by  $\alpha$ -amylase was due to the structural conformation and/or  
342 the catalytic activity, we used an inactivated  $\alpha$ -amylase from *D. melanogaster* with no change in its  
343 structural conformation. Interestingly this inactivated  $\alpha$ -amylase still induced behavioral activities of *C.*  
344 *flavipes* indicating that the conformation rather than the catalytic activity of  $\alpha$ -amylase is crucial in the  
345 host acceptance process by *C. flavipes*.

346 The  $\alpha$ -amylases of both *D. melanogaster* and *C. suppressalis* induced action potentials from the  
347 gustatory neurons of sensilla chaetica located at the tip of antennae of *C. flavipes* females (Figs. 4 and  
348 5); they were however weaker than those induced by the oral secretions of *C. partellus*. BSA induced  
349 action potentials equivalent to the control solution.

350

351 **DISCUSSION**

352 In the current study, a compound involved in host acceptance for oviposition by the wasp *C. flavipes*  
353 isolated from the oral secretion of the larval host *C. partellus* was identified as an  $\alpha$ -amylase. In *Pieris*  
354 *brassicae* (L) (Lepidoptera: Pieridae) larvae, the  $\beta$ -glucosidases of the oral secretion causes the release  
355 of VOCs from Brassicaceae plants that attract parasitoids (Mattiacci et al., 1995). Similarly, volicitin  
356 [N-(17-hydroxylinolenoyl)-L-glutamine] a compound present in the oral secretion of *Spodoptera* sp.  
357 (Lepidoptera: Noctuidae) induces the release of maize VOCs that attract parasitoids (Turlings et al.,  
358 1990; Alborn et al., 1997). In our study, although we have not tested yet if this enzyme induces the  
359 release of VOCs that can attract parasitoids, direct perception of the  $\alpha$ -amylase upon contact elicits the  
360 antennation and stinging attempt behaviors of the parasitoid.

361 Although polypeptides and proteins have previously been reported as chemical signals in the host  
362 selection process by hymenopteran parasitoids (Weseloh, 1977; Bénédet et al., 1999; Gauthier et al.,  
363 2004), the definitive identification of such protein or polypeptide has never been achieved.

364  $\alpha$ -amylases are among the important classes of digestive enzymes used by the insects to hydrolyze  
365 starch to oligosaccharides in various plant tissues; thus they play a critical role in insect survival by  
366 providing energy (Franco et al., 2000). They have been found in several insect orders such as  
367 Orthoptera, Hemiptera, Heteroptera, Hymenoptera, Diptera, Lepidoptera and Coleoptera (Kaur et al.,  
368 2014). In Lepidoptera,  $\alpha$ -amylases have variable molecular weights depending on the species  
369 (Sharifloo et al., 2016), which is unexpected since all known sequences of insect amylases predict  
370 roughly the same weight as those of *Drosophila melanogaster*, i.e.  $\approx$  50 kDa (Boer and Hickey, 1986;  
371 Titarenko and Chrispeels, 2000; Maczkowiak and Da Lage, 2006; Pytelkova et al., 2009; Bezerra et al.,  
372 2014; Channale et al., 2016).

373 The  $\alpha$ -amylases tested in our study had a similar molecular weight as those of *D. melanogaster* (51  
374 kDa) (*C. suppressalis*:  $\approx$  50 kDa, *A. oryzae*: 51 kDa and pig: 50 kDa). Interestingly, all these  $\alpha$ -  
375 amylases induced behavioral responses of *C. flavipes*, suggesting that the size of the  $\alpha$ -amylase is  
376 involved. However, a different protein such as BSA with a similar molecular weight was not inducing  
377 any behavioral response suggesting that the conformation of the protein rather than its weight is  
378 involved in host acceptance for oviposition behavior of the parasitoid. In fact, an inactive  $\alpha$ -amylase of  
379 *D. melanogaster* (with a similar conformation of the active  $\alpha$ -amylase) was still inducing behavioral  
380 responses of *C. flavipes*. This indicates that it is the conformation of the  $\alpha$ -amylase rather than its  
381 catalytic site that induces this activity, and suggests that *C. flavipes* can perceive the  $\alpha$ -amylase  
382 through its sensorial equipment.

383 Obonyo et al. (2010a) observed that female parasitoids (including *C. flavipes*) use the tip of their  
384 antennae to recognize and accept their host larvae for oviposition. They identified on the last antennal  
385 segment the presence of uniporous sensilla chaetica known to have gustatory functions in insects  
386 (Obonyo et al., 2011). Our study confirms that these sensilla chaetica are involved in the perception of  
387 non-volatile host cues as already shown by Iacovone et al. (2016) for the egg parasitoid, *Trissolcus*  
388 *brochymenae* Ashmead (Hymenoptera: Platygasteridae). Gustation in insects is known to be influenced  
389 by small compounds such as sugars, free amino acids, water-soluble alkaloids (see Thiéry et al. [2013]  
390 for review), but the present findings demonstrate that it can also be elicited by larger molecular weight  
391 compounds such as proteins. In addition, as no action potential was generated by  $\alpha$ -amylase from  
392 gustatory neurons of antennal sensilla chaetica of *C. flavipes* males (data not shown), such gustatory  
393 perception of  $\alpha$ -amylase is most likely linked to host acceptance for oviposition behavior in *C. flavipes*  
394 females.

395 The implication of  $\alpha$ -amylase in host recognition and thus selection for oviposition by the parasitoid  
396 implies a stable relationship between  $\alpha$ -amylase variability among host larvae species and host  
397 specificity. In the last decade, it was observed that the diversity of Lepidoptera stemborers in Africa is  
398 considerably higher than described earlier (Le Ru et al., 2006a; 2006b) and that most of these  
399 stemborers are specialists (monophagous, oligophagous), exhibiting a strong host plant conservatism  
400 (Le Ru et al., 2006a; 2006b; Ong'amo et al., 2006a; 2006b; Otieno et al., 2006). In parallel, Mailafiya  
401 et al. (2009) found a higher diversity of the associated parasitoids than previously thought among  
402 *Busseola* spp. and *Chilo* spp. host genera, with an apparent strong host insect conservatism. The  
403 sequences of  $\alpha$ -amylase gene (*Amy*) of a number of animals show a high level of protein variability  
404 (Da Lage et al., 2002). Therefore, the diversity of  $\alpha$ -amylase proteins and of the corresponding *Amy*  
405 genes family may have adaptive or functional significance, for example, in the diversity of stem borers  
406 – parasitoids interactions. In fact, we observed a clearer and stronger behavioral response of *C. flavipes*  
407 with the oral secretion of *C. partellus* containing the genuine  $\alpha$ -amylase than with all the other tested  
408 amylases.

409 A question therefore arises on how the parasitoids access  $\alpha$ -amylase in nature? In fact, Lepidoptera  
410 stemborers larvae spend their life and feed inside plant stems. Before it enters into feeding tunnel of  
411 the host larvae, the wasp makes first contact with the fecal pellets left by the larvae pushed outside of  
412 the stem. Although, these pellets do not induce oviposition, they act as a marker of the status of the  
413 larva inside the stem tunnel as being host or non-host (Obonyo et al., 2010b) and if the host is still  
414 actively feeding or not. However, only when the parasitoid is in contact with the host body, it is able to  
415 recognize and accept it for oviposition (Obonyo et al., 2010a; 2010b). It is during this final step that

416 the parasitoid can access the stimulatory compounds present on the body of the larvae deposited by  
417 their feeding activity. These stimulatory compounds need to give quick and appropriate information to  
418 the parasitoid on the suitability of the larva (both host and health status) because host larvae often bite  
419 the attacking wasps, causing a 50% mortality risk (Takasu and Overholt, 1997). The high selection  
420 pressure due to the high mortality at oviposition should favor wasps that are able to recognize their  
421 hosts with minimal risk of injury (Ward, 1992). Among the stimulatory compounds, this study shows  
422  $\alpha$ -amylases as good candidates for an evolutionary solution to host selection in parasitoids, opening  
423 new routes of investigation in hosts-parasitoids interactions.

424

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430

#### 431 **Competing interests**

432 The authors declare no competing or financial interests.

433

#### 434 **Author contributions**

435 P.-A.C. designed and supervised the study. G.B. carried out all the behavioral experiments, the electrophoresis and the  
436 isolation of proteins for identification. J.-L.D.L. synthesized the  $\alpha$ -amylases from *Drosophila melanogaster* and *Chilo*  
437 *suppressalis* as well as the inactive  $\alpha$ -amylase from *D. melanogaster*. J.-L.D.L. and C.C.-D. realized the western blot  
438 analysis. C.-M. M. performed the electrophysiological experiments. F.M.-P. supervised the electrophysiological  
439 experiments. M.Z. and T.B. realized the protein identification by LC-MS/MS. G.J., E.M. participated to the supervision of  
440 the study. G.B., B.L.R., L.K.-A., G.J., E.M. and P.-A.C. prepared the manuscript. L.K.-A. coordinated the research  
441 program hosting this work.

442

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446

#### 447 **Supplementary information**

448 **Table S1.** Results of proteins and peptides obtained by X!Tandem as well as proteins and peptides obtained by *de novo*  
449 (see attached excel table).

450 **Figure S1.** Map and sequence of the *Chilo suppressalis* 108827 amylase gene construct in the pPICZ-A expression vector  
451 (Invitrogen). The original signal peptide was replaced by the one of *Drosophila melanogaster* amylase. Two restriction  
452 sites were destroyed in the sequence to allow the use of those sites as cloning sites.



453

454 **References**

- 455 **Aghajari, N., Roth, M. and Haser, R.** (2002). Crystallographic evidence of a transglycosylation reaction: ternary  
456 complexes of a psychrophilic  $\alpha$ -amylase. *Biochemistry* **41**, 4273-4280.
- 457 **Alborn, H. T., Turlings, T. C. J., Jones, T. H., Stenhagen, G., Loughrin, J. H. and Tumlinson, J. H.** (1997). An  
458 elicitor of plant volatiles from beet armyworm oral secretion. *Science* **276**, 945-949.
- 459 **B enedet, F., Bigot, Y., Renault, S., Pouzat, J. and Thibout, E.** (1999). Polypeptides of *Acrolepiopsis assectella* cocoon  
460 (Lepidoptera: Yponomeutoidea): an external host-acceptance kairomone for the parasitoid *Diadromus pulchellus*  
461 (Hymenoptera Ichneumonidae). *J. Insect Physiol.* **45**, 375-384.
- 462 **Bezerra, C. A., Macedo, L. L. P., Amorim, T. L. M., Santos, V. O., Fragoso, R. R., Lucena, W. A., Meneguim, A. M.,  
463 Valencia-Jimenez, A., Engler, G., Silva, M. C. M., Albuquerque, E. V. S. and Grossi-de-Sa, M. F.** (2014).  
464 Molecular cloning and characterization of an  $\alpha$ -amylase cDNA highly expressed in major feeding stages of the  
465 coffee berry borer, *Hypothenemus hampei*. *Gene* **553**, 7-16.
- 466 **Boer, P. H. and Hickey, D. A.** (1986). The  $\alpha$ -amylase gene in *Drosophila melanogaster*: nucleotide sequence, gene  
467 structure and expression motifs. *Nucl. Ac. Res.* **14**, 8399-8411.
- 468 **Calatayud, P.-A., Le Ru, B., Schulthess, F. and Silvain, J.-F.** (2006). Research on the lepidopteran graminaceous  
469 stemborers and their antagonists: achievement and perspectives. *Ann. Soc. Entomol. Fr.* **42**, 259-262.
- 470 **Channale, S. M., Bhide, A. J., Yadav, Y., Kashyap, G., Pawar, P. K., Maheshwari, V. L., Ramasamy, S. and Giri, A.  
471 P.** (2016). Characterization of two coleopteran  $\alpha$ -amylases and molecular insights into their differential inhibition by  
472 synthetic  $\alpha$ -amylase inhibitor, acarbose. *Insect Biochem. Molec. Biol.* **74**, 1-11.
- 473 **Chng, W. A., Bou Sleiman, M. S., Sch upfer, F. and Lemaitre, B.** (2014). Transforming growth factor  $\beta$ /activin signaling  
474 functions as a sugar-sensing feedback loop to regulate digestive enzyme expression. *Cell Rep.* **9**, 336-348.
- 475 **Chrambach, A. and Jovin, T. M.** (1983). Selected buffer systems for moving boundary electrophoresis on gels at various  
476 pH values, presented in a simplified manner. *Electrophoresis* **4**, 190-204.
- 477 **Commin, C., Aumont-Nicaise, M., Claisse, G., Feller, G. and Da Lage, J.-L.** (2013). Enzymatic characterization of  
478 recombinant  $\alpha$ -amylase in the *Drosophila melanogaster* species subgroup: is there an effect of specialization on  
479 digestive enzyme? *Genes Genet. Sys.* **88**, 251-259.
- 480 **Craig, R. and Beavis, R. C.** (2004). TANDEM: matching proteins with tandem mass spectra. *Bioinformatics* **20**, 1466-  
481 1467.
- 482 **Da Lage, J.-L., Van Wormhoudt, A. and Cariou, M.-L.** (2002). Diversity and evolution of the  $\alpha$ -amylase genes in  
483 animals. *Biologia Bratislava* 57/Suppl **11**, 181-189.
- 484 **Franco, O. L., Ridgen, D. J., Melo, F. R., Bloch, C. Jr., Silva, C. P. and Grossi-de-Sa, M. F.** (2000). Activity of wheat  
485  $\alpha$ -amylase inhibitors towards bruchid  $\alpha$ -amylases and structural explanation of observed specificities. *Eur. J.*  
486 *Biochem.* **267**, 2166-2173.
- 487 **Gauthier, N., Mandon, N., Renault, S. and B enedet, F.** (2004). The *Acrolepiopsis assectella* silk -cocoon: kairomonal  
488 function and chemical characterization. *J. Insect Physiol.* **50**, 1065-1074.
- 489 **Godfray, H. C. J., Beddington, J. R., Crute, I. R., Haddad, L., Lawrence, D., Muir, J. F., Pretty, J., Robinson, S.,  
490 Thomas, S. M. and Toulmin, C.** (2010). Food security: The challenge of feeding 9 billion people. *Science* **327**,  
491 812-818.
- 492 **Harris, K. M.** (1990). Bioecology of sorghum stemborers. *Insect Sci. Appl.* **11**, 467-477.
- 493 **Hawkins, B. A.** (1994). *Pattern and process in host-parasitoid interactions* (Cambridge University Press, Cambridge).
- 494 **Hodgson, E. S., Lettvin, J. Y. and Roeder, K. D.** (1955). Physiology of a primary chemoreceptor unit. *Science* **122**, 417-  
495 418.

- 496 **Iacovone, A., French, A. S., Tellier, F., Cusumano, A., Clément, G., Gaertner, C., Conti, E., Salerno, G. and Marion-**  
497 **Poll, F.** (2016). The role of contact chemoreception in host location process of an egg parasitoid. *J. Insect Physiol.*  
498 **91-92**, 63-75.
- 499 **Inayatullah, C.** (1983). Host selection by *Apanteles flavipes* (Cameron) (Hymenoptera: Braconidae): influence of host and  
500 host plant. *J. Econ. Entomol.* **76**, 1086-1087.
- 501 **Jiang, N., Zhou, G., Overholt, W. A. and Schulthess, F.** (2006). The synchrony of the stem borer and parasitoid  
502 populations of coastal Kenya. *Ann. Soc. Entomol. Fr.* **42**, 381-388.
- 503 **Kaiser, L., Dupas, S., Branca, A., Herniou, E. A., Clarke, C. W., Capdevielle-Dulac, C., Obonyo, J., Benoist, R.,**  
504 **Gauthier, J., Calatayud, P.-A., Silvain, J.-F. and Le Ru, B. P.** (2017a). The *Cotesia sesamiae* story: insight into  
505 host-range evolution in a Hymenoptera parasitoid and implication for its use in biological control programs.  
506 *Genetica* **145**, 455-468.
- 507 **Kaiser, L., Ode, P., van Nouhuys, S., Calatayud, P.-A., Colazza, S., Cortesero, A.-M., Thiel, A. and van Baaren, J.**  
508 (2017b). The Plant as a Habitat for Entomophagous Insects. *Insect-Plant Interactions in a Crop Protection*  
509 *Perspective*, eds Sauvion, N., Calatayud, P.-A. & Thiéry, D. (Elsevier, GBR) Advances in Botanical Research series,  
510 Vol 81, pp 179-224. doi:10.1016/bs.abr.2016.09.006.
- 511 **Kaur, R., Kaur, N. and Gupta, A. K.** (2014). Structural features, substrate specificity kinetic properties of insect  $\alpha$ -  
512 amylase and specificity of plant  $\alpha$ -amylase inhibitors. *Pestic. Biochem. Physiol.* **116**, 83-93.
- 513 **Kfir, R.** (1992). Seasonal abundance of the stem borer *Chilo partellus* (Lepidoptera: Pyralidae) and its parasites on summer  
514 grain crops. *J. Econ. Entomol.* **85**, 518-529.
- 515 **Kfir, R.** (1995). Parasitoids of the African stem borer *Busseola fusca* (Lepidoptera: Noctuidae) in South Africa. *Bull.*  
516 *Entomol. Res.* **85**, 369-377.
- 517 **Kfir, R., Overholt, W. A., Khan, Z. R. and Polaszek, A.** (2002). Biology and management of economically important  
518 lepidopteran cereal stem borers in Africa. *Annu. Rev. Entomol.* **47**, 701-731.
- 519 **Kurien, B. T. and Scofield, R. H.** (2012). Extraction of proteins from gels: A brief review. *Methods Mol. Biol.* **869**, 403-  
520 405.
- 521 **Langella, O., Valot, B., Balliau, T., Blein-Nicolas, M., Bonhomme, L. and Zivy, M.** (2016). X! TandemPipeline: a tool  
522 to manage sequence redundancy for protein inference and phosphosite identification. *J. Proteome Res.* **16**, 494-503.
- 523 **Lazarovitz, G., Goettel, S. and Vincent, C.** (2007). Adventures in biological control. *Biological Control: A Global*  
524 *Perspective: Case Studies from Around the World*, eds Vincent C, Goettel S, Lazarovitz G (CABI, Wallingford), pp.  
525 1-6.
- 526 **Le Ru, B. P., Ong'amo, G. O., Moyal, P., Muchugu, E., Ngala, L., Musyoka, B., Abdullah, Z., Matama-Kauma, T.,**  
527 **Lada, V. Y., Pallangyo, B., Omwega, C. O., Schulthess, F., Calatayud, P.-A. and Silvain, J.-F.** (2006a).  
528 Geographic distribution and host plant ranges of East African noctuid stem borers. *Ann. Soc. Entomol. Fr.* **42**, 353-  
529 361.
- 530 **Le Ru, B. P., Ong'amo, G. O., Moyal, P., Ngala, L., Musyoka, B., Abdullah, Z., Cugala, D., Defabachew, B., Haile, T.**  
531 **A., Matama-Kauma, T., Lada, V. Y., Negassi, B., Pallangyo, B., Ravololonandrianina, J., Sidumo, A.,**  
532 **Omwega, C. O., Schulthess, F., Calatayud, P.-A. and Silvain, J.-F.** (2006b). Diversity of lepidopteran stem borers  
533 on monocotyledonous plants in eastern Africa and island of Madagascar and Zanzibar revisited. *Bull. Entomol. Res.*  
534 **96**, 1-9.
- 535 **Mackey, A. J., Haystead, T. A. and Pearson, W. R.** (2002). Getting more from less algorithms for rapid protein  
536 identification with multiple short peptide sequences. *Mol. Cell. Proteomics* **1**, 139-147.
- 537 **Maczkowiak, F. and Da Lage, J.-L.** (2006). Origin and evolution of the *Amyrel* gene in the  $\alpha$ -amylase family of  
538 Diptera. *Genetica* **128**, 145-158.
- 539 **Mailafiya, D. M., Le Ru, B. P., Kairu, E. W., Calatayud, P.-A. and Dupas, S.** (2009). Species diversity of lepidopteran  
540 stem borer parasitoids in cultivated and natural habitats in Kenya. *J. Appl. Entomol.* **133**, 416-429.
- 541 **Marascuilo, L.** (1966). Large-sample multiple comparisons. *Psychol. Bull.* **65**, 289-299.

- 542 **Marion-Poll, F. and van der Pers, J.** (1996). Un-filtered recordings from insect taste sensilla. *Entomol. Exp. Appl.* **80**,  
543 113–115.
- 544 **Marion-Poll, F.** (1996). Display and analysis of electrophysiological data under MSWindows. *Entomol. Exp. Appl.* **80**,  
545 116–119.
- 546 **Mason, W. R. M.** (1981). The polyphyletic nature of *Apanteles* Foerster (Hymenoptera: Braconidae): a phylogeny and  
547 reclassification of Microgastrinae. *Mem. Entomol. Soc. Can.* **115**, 1-147.
- 548 **Mattiacci, L., Dicke, M. and Posthumus, M. A.** (1995). beta-Glucosidase: an elicitor of herbivore-induced plant odor that  
549 attracts host-searching parasitic wasps. *PNAS* **92**, 2036-2040.
- 550 **Mohyuddin, A. I., Inayatullah, C. and King, E. G.** (1981). Host selection and strain occurrence in *Apanteles flavipes*  
551 (Cameron)(Hymenoptera: Braconidae) and its rearing on biological control of graminaceous stem-borers  
552 (Lepidoptera: Pyralidae). *Bull. Entomol. Res.* **71**, 575-581.
- 553 **Ngi-Song, A. J. and Overholt, W. A.** (1997). Host location and acceptance of *Cotesia flavipes* Cameron and *Cotesia*  
554 *sesamiae* (Cameron) (Hymenoptera : Braconidae), parasitoids of African stemborers : role of frass and other cues.  
555 *Biol. Control* **9**, 136-142.
- 556 **Niepmann, M. and Zheng, J.** (2006). Discontinuous native protein gel electrophoresis. *Electrophoresis* **27**, 3949–3951.
- 557 **Obonyo, M., Schulthess, F., Juma, G., Wanyama, O., Le Ru, B. and Calatayud, P.-A.** (2008). Location, acceptance  
558 and suitability of lepidopteran stemborers feeding on a cultivated and wild host-plant to endoparasitoid *Cotesia*  
559 *flavipes* Cameron (Hymenoptera : Braconidae). *Biol. Control* **45**, 36-47.
- 560 **Obonyo, M., Schulthess, F., Le Ru, B., Van Den Berg, J. and Calatayud, P.-A.** (2010a). Host recognition and  
561 acceptance behaviour in *Cotesia sesamiae* and *C. flavipes* (Hymenoptera: Braconidae), parasitoids of gramineous  
562 stemborers in Africa. *Eur. J. Entomol.* **107**, 169–176.
- 563 **Obonyo, M., Schulthess, F., Le Ru, B., Van Den Berg, J., Silvain, J.-F. and Calatayud, P.-A.** (2010b). Importance of  
564 contact chemical cues in host recognition and acceptance by the braconid larval endoparasitoids *Cotesia sesamiae*  
565 and *Cotesia flavipes*. *Biol. Control* **54**, 270–275.
- 566 **Obonyo, M., Schulthess, F., Chintawi, M., Mascarel, G., Ahuya, P. O., Le Ru, B., Van Den Berg, J., Silvain, J.-F.**  
567 **and Calatayud, P.-A.** (2011). Sensilla on antennae, ovipositor and tarsi of the larval parasitoids, *Cotesia sesamiae*  
568 (Cameron 1906) and *Cotesia flavipes* Cameron 1891 (Hymenoptera: Braconidae): a comparative scanning electron  
569 microscopy study. *Ann. Soc. Entomol. Fr.* **47(1-2)**, 119–127.
- 570 **Ochieng, R. S., Onyango, F. O. and Bungu, M. D. O.** (1985). Improvement of techniques for mass culture of *Chilo*  
571 *partellus* (Swinhoe). *Insect Sci. Appl.* **6**, 425–428.
- 572 **Ong'amo, G. O., Le Ru, B. P., Dupas, S., Moyal, P., Calatayud, P.-A. and Silvain, J.-F.** (2006a). Distribution, pest  
573 status and agro-climatic preferences of lepidopteran stem borers of maize in Kenya. *Ann. Soc. Entomol. Fr.* **42(2)**,  
574 171–177.
- 575 **Ong'amo, G., Le Ru, B. P., Dupas, S., Moyal, P., Muchugu, E., Calatayud, P.-A. and Silvain, J.-F.** (2006b). The role  
576 of wild host plants in the abundance of lepidopteran stem borers along altitudinal gradient in Kenya. *Ann. Soc.*  
577 *Entomol. Fr.* **42**, 363-370.
- 578 **Otieno, N. A., Le Ru, B. P., Ong'amo, G. O., Dupas, S., Calatayud, P.-A., Makobe, M., Ochora, J. and Silvain, J.-F.**  
579 (2006). Diversity and abundance of wild host plants of lepidopteran stem borers in two different agroecological  
580 zones of Kenya. *Ann. Soc. Entomol. Fr.* **42(3–4)**, 371–380.
- 581 **Overholt, W. A., Ochieng, J. O., Lammers, P. M. and Ogedah, K.** (1994). Rearing and field release methods for *Cotesia*  
582 *flavipes* Cameroon (Hymenoptera: Braconidae), a parasitoid of tropical gramineous stemborers. *Insect Sci. Appl.* **15**,  
583 253–259.
- 584 **Pimentel, D., Acquay, H., Biltonen, M., Rice, P., Silva, M., Nelson, J., Lipner, V., Giordano, S., Horowitz, A. and**  
585 **D'Amore, M.** (1992). Environmental and economic costs of pesticide use. *BioScience* **42**, 750–760.
- 586 **Polaszek, A.** (1998). *African Cereal Stemborers: Economic Importance, Taxonomy, Natural Enemies and Control* (CABI,  
587 Wallingford, Oxon, United Kingdom).

- 588 **Potting, R. P. J., Osaе-Danso, F., Overholt, W. A. and Ngi-Song, A. J.** (1993). Host selection in *Cotesia flavipes*,  
589 parasitoid of tropical stemborers. *Proc. Exp. Appl. Entomol.* **4**, 47-52.
- 590 **Pytelkova, J., Hubert, J., Lepsik, M., Sobotnik, J., Sindelka, R. and Krizkova, I.** (2009). Digestive  $\alpha$ -amylases of the  
591 flour moth *Ephestia kuehniella*-adaptation to alkaline environment and plant inhibitors. *FEBS J.* **276**, 3531-3546.
- 592 **Sallam, N. M., Overholt, W. A. and Kairu, E.** (1999). Comparative evaluation of *Cotesia flavipes* and *C. sesamiae*  
593 (Hymenoptera: Braconidae) for the management of *Chilo partellus* (Lepidoptera: Pyralidae) in Kenya. *Bull.*  
594 *Entomol. Res.* **89**, 185–191.
- 595 **Sharifloo, A., Zibae, A., Sendi, J. J. and Jahroumi, K. T.** (2016). Characterization of a digestive  $\alpha$ -amylase in the  
596 midgut of *Pieris brassicae* L. (Lepidoptera: Pieridae). *Front. Physiol.* **7**, 96 doi: 10.3389/fphys.2016.00096
- 597 **Smith, P. K., Krohn, R. I., Hermanson, G. T., Mallia, A. K., Gartner, F. H., Provenzano, M., Fujimoto, E. K., Goeke,  
598 N. M., Olson, B. J. and Klenk, D. C.** (1985). Measurement of protein using bicinchoninic acid. *Anal. Biochem.* **150**,  
599 76-85.
- 600 **Songa, J. M., Jiang, N., Schulthess, F. and Omwega, C.** (2007). The role of intercropping different cereal species in  
601 controlling lepidopteran stemborers on maize in Kenya. *J. Appl. Entomol.* **131**, 40–49.
- 602 **Takasu, K. and Overholt, W. A.** (1997). Aggressive behaviour of *Chilo partellus* (Swinhoe) larvae against the parasitoid  
603 *Cotesia flavipes* Cameron. *Insect Sci. Appl.* **17**, 131–136.
- 604 **Thiéry, D., Derridj, S., Calatayud, P.-A., Nevile, M. and Marion-Poll, F.** (2013). L'insecte au contact des plantes. pp :  
605 347-368 dans « Interactions insectes – plantes », Sauvion, N., Calatayud, P.-A., Thiéry, D. and Marion-Poll, F.  
606 (Eds.), Co-édition IRD-Quae, Paris.
- 607 **Tilman, D., Fargione, J., Wolff, B., D'Antonio, C., Dobson, A., Howarth, R., Schindler, D., Schlesinger, W. H.,  
608 Simberloff, D. and Swackhamer, D.** (2001). Forecasting agriculturally driven global environmental change.  
609 *Science* **292**, 281–284.
- 610 **Titarenko, E. and Chrispeels, M. J.** (2000). cDNA cloning, biochemical characterization and inhibition by plant  
611 inhibitors of the  $\alpha$ -amylases of the Western corn rootworm, *Diabrotica virgifera virgifera*. *Insect Biochem. Molec.*  
612 *Biol.* **30**, 979-990.
- 613 **Turlings, T. C. J., Tumlinson, J. H. and Lewis, W. J.** (1990). Exploitation of herbivore-induced plant odors by host  
614 seeking parasitic wasps. *Science* **250**, 1251-1253.
- 615 **Van Leerdam, M. B., Smith, J. W. Jr. and Fuchs, T. W.** (1985). Frass-mediated, host finding behaviour of *Cotesia*  
616 *flavipes*, a braconid parasite of *Diatraea saccharalis* (Lepidoptera: Pyralidae). *Ann. the Entomol. Soc. Am.* **78**, 647-  
617 650.
- 618 **Wajnberg, E., Bernstein, C. and Van Alphen, J.** (2008). *Behavioral of ecology of insect parasitoids* (Blackwell  
619 Publishing, USA, UK, Australia).
- 620 **Wajnberg, E. and Colazza, S.** (2013). *Chemical ecology of insect parasitoids* (Wiley-Blackwell, A John Wiley & Sons,  
621 Ltd., Publication, UK).
- 622 **Ward, S. A.** (1992). Environmental uncertainty and polyphagy in herbivorous insects. *Oikos* **63**, 506–512.
- 623 **Weseloh, R. M.** (1977). Effects on behavior of *Apanteles melanoscelus* females caused by modifications in extraction,  
624 storage and presentation of gypsy moth silk kairomone. *J. Chem. Ecol.* **3**, 723-735.
- 625 **Wieczorek, H. and Wolff, G.** (1989). The labellar sugar receptor of *Drosophila*. *J. Comp. Physiol. A* **164**, 825-834.
- 626 **Yu, D. S. K., Van Achterberg, C. and Horstmann, K.** (2016). Taxapad 2016. Ichneumonoidea 2015. Database on flash-  
627 drive. Nepean, Ontario, Canada. doi: <http://www.taxapad.com>
- 628 **Zhou, G., Baumgartner, J. and Overholt, W. A.** (2001). Impact of an exotic parasitoid on stemborer (Lepidoptera)  
629 population dynamics in Kenya. *Ecol. Entomol.* **11**, 1554–1561.

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635 **Table 1. Response of *Cotesia flavipes* parasitic wasps to oral secretions of its host, larva of *Chilo***  
636 ***partellus***

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Behaviours of the parasitoid *Cotesia flavipes*

| Type of sample  |  |
|---|--|
|   | % antennation + stinging attempt<br>(n=20)   |
| Oral secretion of larvae fed on <i>Zea mays</i> stems               | 90b  |
| Oral secretion of larvae fed on <i>Pennisetum purpureum</i> stems   | 87b  |
| Oral secretion of larvae fed on artificial diet                     | 0a   |
| Oral secretion of starved larvae                                    | 0a   |
| Oral secretion of larvae fed on maize stems treated by proteinase K | 0a   |

638 % followed by different letters are significantly different at 5% level (Marascuilo's procedure).

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648 **Table 2. Response of *Cotesia flavipes* parasitic wasps to the seven main electrophoretic bands (see**  
649 **Figure 1) obtained from the oral secretions of its host, larva of *Chilo partellus***

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Behaviours of the parasitoid *Cotesia flavipes*



| Band tested | % antennation + stinging attempt<br>(n=10) |
|-------------|--|
| 1           | 0a   |
| 2           | 0a   |
| 3           | 30a  |
| 4           | 90b  |
| 5           | 0a   |
| 6           | 0a   |
| 7           | 0a   |

651 % followed by different letters are significantly different at 5% level (Marascuilo's procedure).

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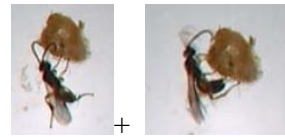
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664 **Table 3. Response of *Cotesia flavipes* parasitic wasps to purified proteins (at 300-500 ng/μl) from**  
 665 **different origins**

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Behaviours of the parasitoid *Cotesia flavipes*

| Proteins tested  | antennation + stinging attempt<br>(mean* ± SE, n=6) |
|--|---|
| A-amylase from <i>Aspergillus oryzae</i>               | 43.3 ± 6.1bc  |
| A-amylase from pig                                     | 20.0 ± 7.3ab  |
| A-amylase from <i>Drosophila melanogaster</i>          | 70.0 ± 6.8d   |
| A-amylase from <i>Chilo suppressalis</i>               | 60.0 ± 7.3cd  |
| Inactive α-amylase from <i>Drosophila melanogaster</i> | 53.3 ± 6.7cd  |
| BSA  | 0a  |



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\*Means with different letter are significant (q-value <0.05; pairwise Wilcoxon's rank sum test, q-value = FDR corrected p-value).

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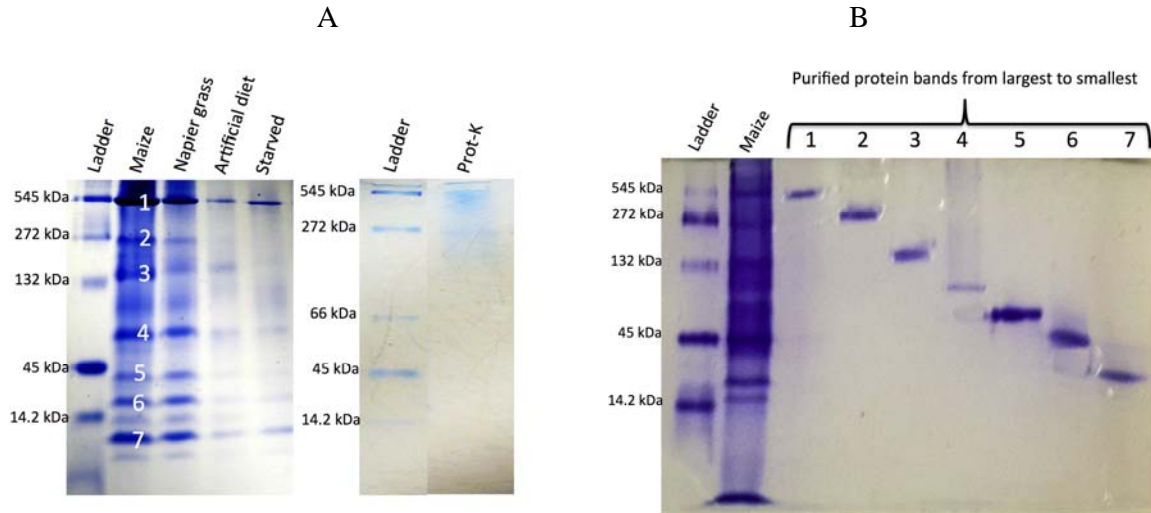
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684 **Fig. 1. Analysis of oral extracts in a native gel system.** Protein samples were separated by 1D gel,  
685 7% native Onstein-Davis discontinuous (Tris-glycine) PAGE before Coomassie staining.

686 A) Comparison of *Chilo partellus* oral extract fed on different diet. Ladder: Sigma molecular weight  
687 markers; lane 1: oral secretion from *Chilo partellus* larvae fed on maize stems (Maize)(each main  
688 electrophoretic band [noted 1 to 7 on the gel] were individually extracted from the gel (see Fig. 1B)  
689 under non-denaturing conditions and tested towards *Cotesia flavipes* (see Table 2); lane 2: oral  
690 secretion from *Chilo partellus* larvae fed on *Pennisetum purpureum* stems (Napier grass); lane 3: oral  
691 secretion from *Chilo partellus* larvae fed on artificial diet (Artificial diet); lane 4: oral secretion from  
692 starved larvae of *Chilo partellus* (Starved). For each lane, 15 $\mu$ l of the oral secretion was loaded after  
693 concentrating and before quantification of the samples (Bio Rad Mini-PROTEAN® Electrophoresis  
694 System). After proteinase K treatment no band was obtained (Prot-K).

695 B) Individual protein band purified from the gel of regurgitant of *Chilo partellus* fed on maize. Lanes:  
696 1 molecular weight marker (sigma Aldrich), 2 regurgitants from *Chilo partellus* fed on maize (Maize);  
697 lanes 1-7 bands purified and tested for activity against *Cotesia flavipes* (Hofer™ Mini Vertical  
698 Electrophoresis Systems (Fisher Sci.com) (see Table 2).

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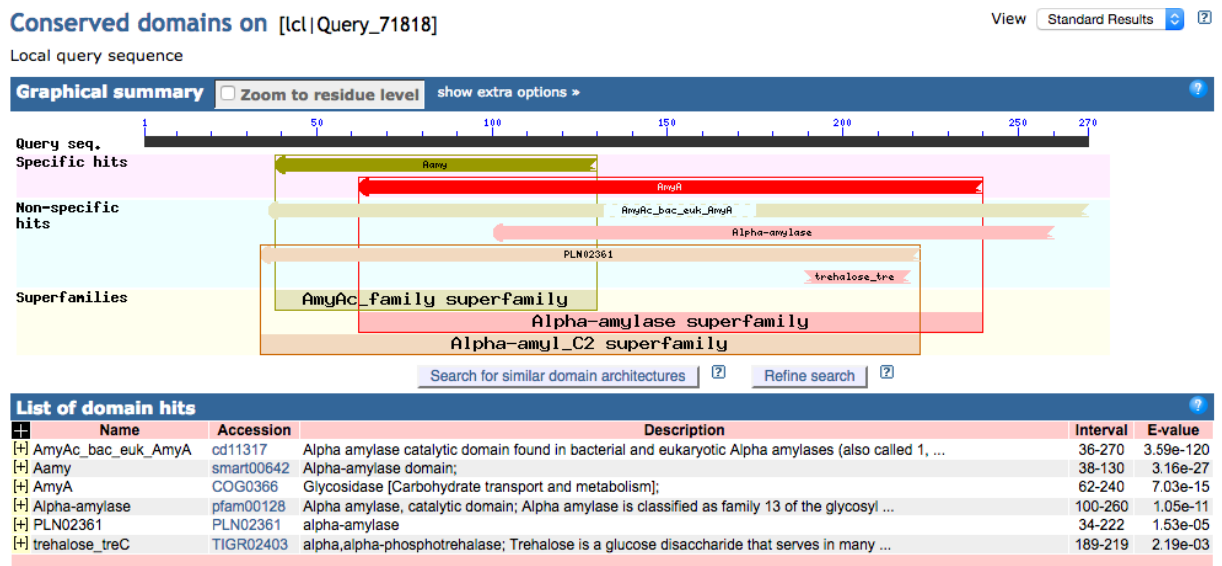
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710 >gi|295290041|gb|FP379314.1|FP379314|Frame3 FP379314 Spodoptera frugiperda cDNA library,  
711 induced midguts Spodoptera frugiperda cDNA clone Sf2M05200-5-1, mRNA sequence

712 VIVHGVISVRMFRLILCLAAVTLALAYKNPHYASGRRTMVHLFEWKWDDIARECETFLG  
713 PRGYGGIQUISPPNENLAIWSRQRPWERYQPISYRLVTRSGNEQQFANMVRKCNDAGVRI  
714 YVDAIINHMTGTWNENTGTGGSTADFGNWGYPGVPYGRNDFNWPVCVIQGHDIYGCCADRV  
715 RNCELSGLKDLNQGNEYVRQQIVNYMNHNLINLGVAGFRIDAAKHMWPGDLRVIYDRLHNL  
716 NTAHGFPSGARPYIYQEVIDLGGEIISRDEY  
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720 **Fig. 2. Protein-protein BLAST result of the *de novo* protein sequence.** A) The best *de novo* protein  
721 sequence associated with EST specific to *Spodoptera frugiperda* database (see Table S1). B) The pre-  
722 computed domain annotation for the best *de novo* protein sequence of A) using the protein data bank  
723 of the BLAST ® online software (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). The section circled in red  
724 provides the functional label that has been assigned to the subfamily domain.

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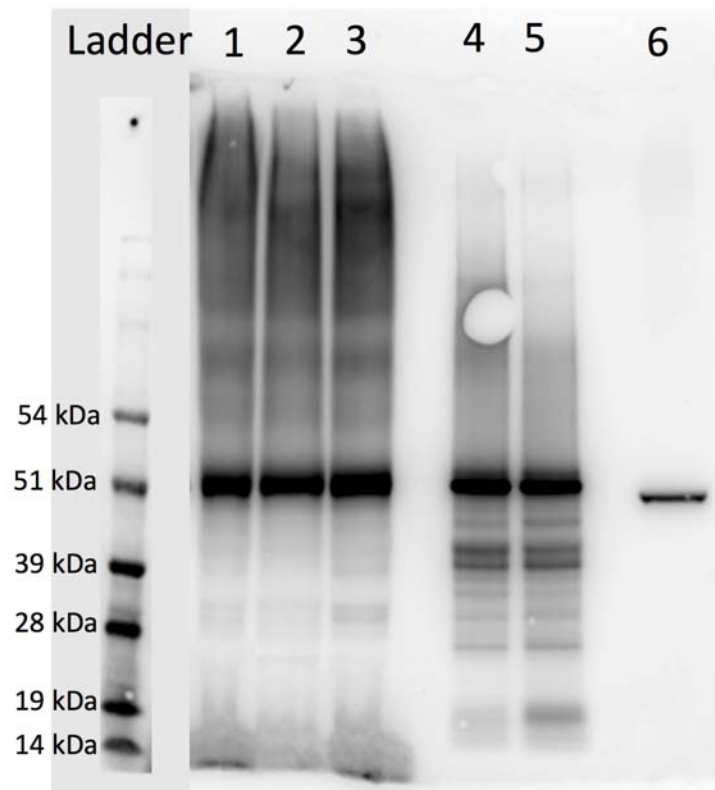
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734 **Fig. 3. Western blot performed with an antibody specific to *Drosophila melanogaster*  $\alpha$ -amylase.**  
735 Ladder: molecular weight markers (pre-stained SeeBlue Plus2, Thermo Fischer); 1, 2 and 3: oral  
736 secretions from *Chilo partellus* larvae fed on maize stems; 4 and 5: band n°4 of Fig. 1 which has been  
737 extracted from the gel and used for Western Blot analysis; 6:  $\alpha$ -amylase from *Drosophila*  
738 *melanogaster*.

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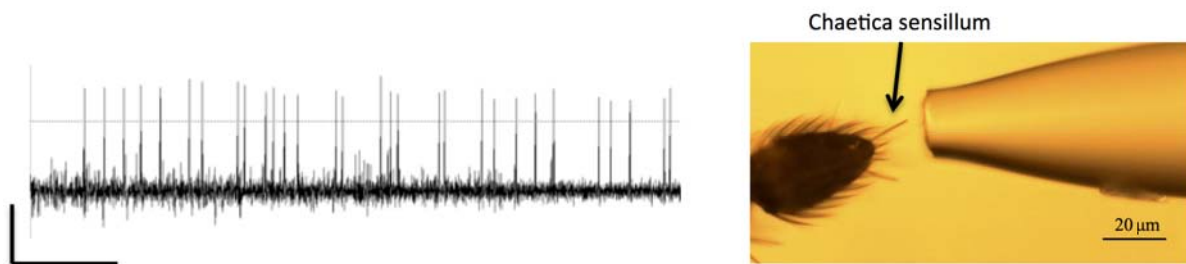
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760 **Fig. 4.** Left: 2 s chemosensory recording is displayed showing the response of a chaetia sensillum at  
761 the tip of *Cotesia flavipes* antennal female to  $\alpha$ -amylase of *Drosophila melanogaster* (at 300 ng/ $\mu$ l).  
762 Vertical bar: 2 mV; horizontal bar: 200ms. Right: Photo of the tip of an antenna stimulated by a  
763 capillary electrode.

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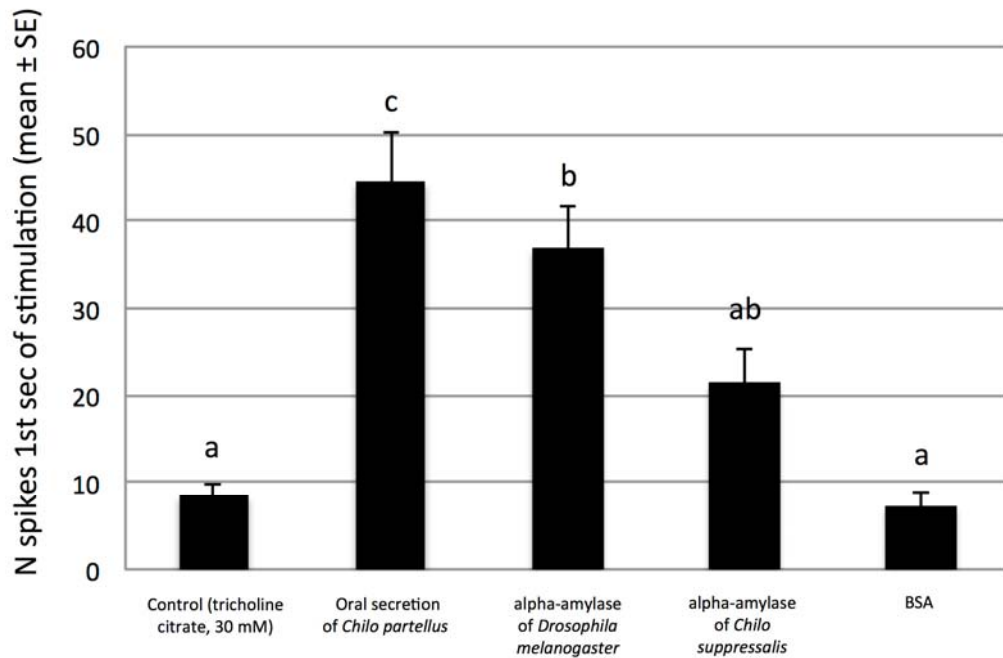
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781 **Fig. 5. Electrophysiological responses of *Cotesia flavipes* females to oral secretion of *Chilo***  
782 ***partellus* and to different proteins (at 300 ng/ $\mu$ l).** The recordings were made on sensilla chaetica  
783 located at the apical antennal segments of *Cotesia flavipes* females. Each bar represents the mean ( $\pm$   
784 SE, n=10) number of action potentials during the first second of stimulation. Different letters capping  
785 the bars indicate significant differences ( $P < 0.05$ ) among mean responses elicited by the different  
786 stimuli (one-way ANOVA, Tukey's contrasts test).

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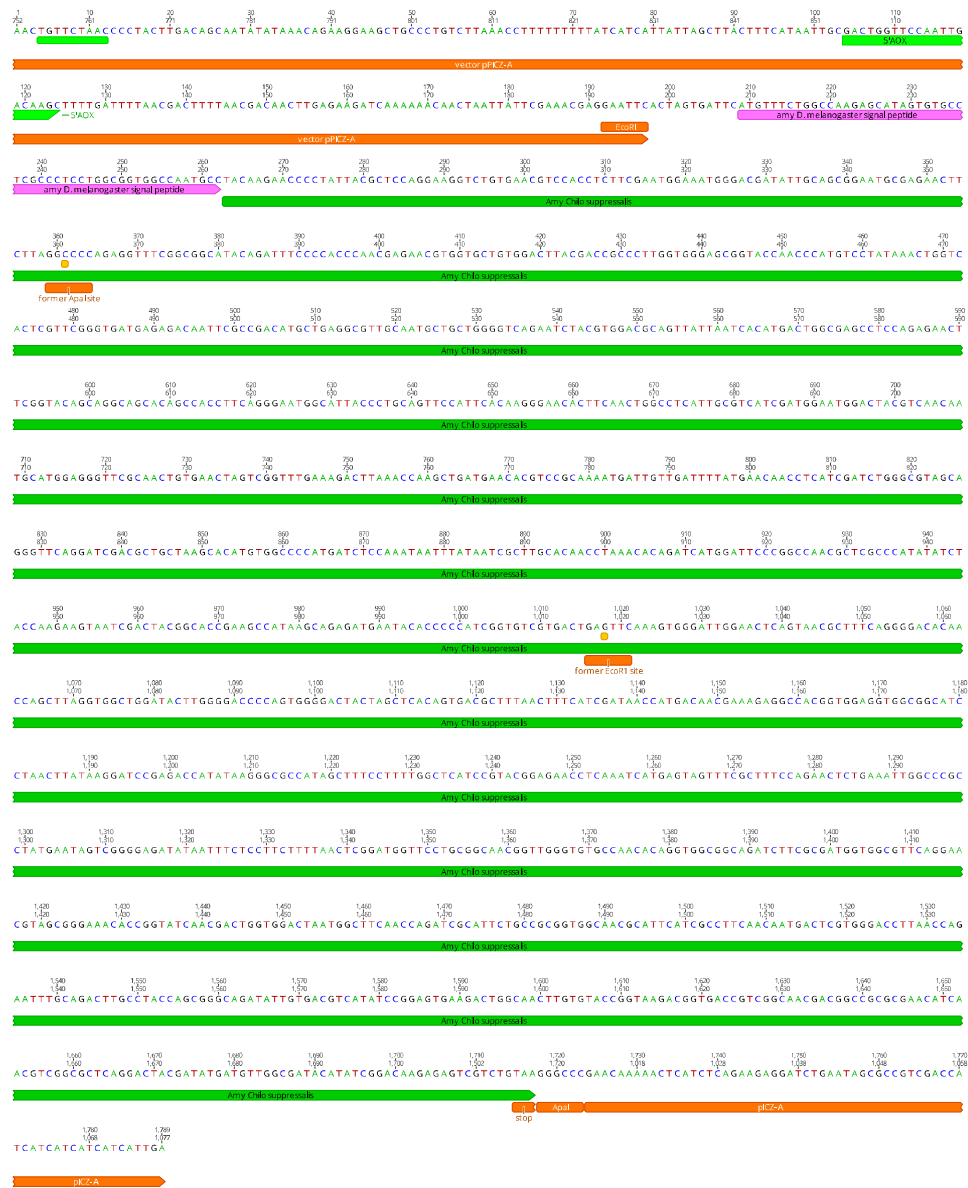
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## Supplementary Table and Figure



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798 **Fig. S1. Map and sequence of the *Chilo suppressalis* 108827 amylase gene construct in the**  
 799 **pPICZ-A expression vector (Invitrogen). The original signal peptide was replaced by the one of**  
 800 ***Drosophila melanogaster* amylase. Two restriction sites were destroyed in the sequence to allow the**  
 801 **use of those sites as cloning sites.**

802 **Table S1. Results of proteins and peptides obtained by X!Tandem as well as proteins and**  
 803 **peptides obtained by *de novo* (see attached excel table).**