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Sucrose taste receptors differ in larval and adult stages of a moth

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Abstract

Almost all herbivorous insects feed on plants and use sucrose as a feeding stimulant, but the molecular basis of their sucrose reception remains unclear. The cotton bollworm *Helicoverpa armigera* as a notorious crop pest worldwide mainly feeds on reproductive organs of many plant species in the larval stage, and its adult draws nectar. Its gustatory sensory neurons sensitive to sucrose are distributed in the taste sensilla of larval maxillary galeas and adult antennae, tarsi and proboscis. In this study, using the ectopic expression system, we discovered that *Gr10* highly expressed in the larval sensilla was specifically tuned to sucrose, while *Gr6* highly expressed in the adult sensilla responded to fucose, sucrose and fructose. Moreover, using the CRISPR/Cas9 technique, we revealed that *Gr10* was mainly used by larvae to detect lower sucrose, while *Gr6* was primarily used by adults to detect higher sucrose and other saccharides, which results in the differences in selectivity and sensitivity between larval and adult sugar sensory neurons. Our results demonstrate the sugar receptors in this moth are evolved to adapt towards the larval and adult foods with different types and amounts of sugar, and fill in a gap in sweet taste of animals.

Keywords: sucrose, gustatory receptor, cotton bollworm, CRISPR/Cas9, *Xenopus* expression and two-electrode voltage-clamp

Introduction

Over 400,000 species of phytophagous insects live on approximately 300,000 species of vascular plants worldwide (Schoonhoven et al., 2005). Carbohydrates produced by plant photosynthesis are important nutrients for phytophagous insects. The disaccharide sucrose, the major sugar in plants, is the principal transport form of photoassimilates in higher plants. Sucrose varies considerably in concentration in green leaves, fruits, and flower nectar where it occurs together with its constituent monosaccharides fructose and glucose (Bernays & Chapman, 1994). Sucrose, fructose and glucose are the powerful phagostimulants for most phytophagous insects studied (Bernays & Chapman, 1994; Blaney & Simmonds, 1988; Chapman, 2003; Schoonhoven et al., 2005).

The gustatory sensory neurons (GSNs) in one or more mouthpart sensilla of phytophagous insects respond to sugars, usually including sucrose (Chapman, 2003; Dethier, 1973). In Lepidoptera, caterpillars often have sugar GSNs in lateral and medial sensilla styloconica in the maxillary galea (Glendinning et al., 2009; Martin & Shields, 2012; Schoonhoven & van Loon, 2002), while adults also have sugar GSNs in taste sensilla of the antennae (Jørgensen et al., 2007; Popescu et al., 2013), tarsi (Schnuch & Seebauer, 1998; Zhang et al., 2010), and proboscis (Blaney & Simmonds, 1988; Faucheux, 2013). The molecular basis of sugar reception in Lepidopteran insects remains unclear although the sweet taste receptors in mammals and *Drosophila melanogaster* have been extensively studied (Liman et al., 2014; Yarmolinsky et al., 2009). In mammals, the combination of two taste receptors T1R2 and T1R3 recognizes sugars including sucrose (Damak et al., 2003; Li et al., 2002;

Nelson et al., 2001; Zhao et al., 2003). In *Drosophila*, multiple gustatory receptors (GRs), including DmGr5a, DmGr61a, and DmGr64a–f clusters, are required for the response of adults to multiple sugars (Dahanukar et al., 2001; Dahanukar et al., 2007; Jiao et al., 2007; Jiao et al., 2008; Slone et al., 2007), while DmGr43a specifically responding to fructose is the major larval sugar receptor (Mishra et al., 2013; Miyamoto et al., 2012).

The cotton bollworm *Helicoverpa armigera* (Noctuidae, Lepidoptera) is a worldwide agricultural pest, it causes over $3 billion in annual global economic loss (Pearce et al., 2017). The larvae of *H. armigera* mainly feeds on young leaves, buds, and young fruits of host plants; the adults mainly drink nectar of plants. Larval and adult diets vary dramatically in the variety and concentration of sugars. Sucrose is the major sugar in plant leaves and reproductive organs, whereas flower nectar mainly contains sucrose, fructose and glucose (Nicolson, 1998). Previous studies have shown that GSNs sensitive to sucrose and fructose in *H. armigera* are located in the lateral sensilla styloconica in larvae (Tang et al., 2000; Zhang et al., 2013) and the taste sensilla of antennae (Jiang et al., 2015), tarsi (Blaney & Simmonds, 1990; Zhang et al., 2010), and proboscis (Blaney & Simmonds, 1988) in adults.

To understand the molecular basis of sugar reception in these neurons, we first compared electrophysiological and behavioral responses of *H. armigera* larvae and adults. Second, we analyzed the expression patterns of nine candidate sugar GR genes in larval and adult taste organs. Third, we carried out functional analysis of these GR genes using the ectopic expression system, and revealed the response profiles of *Xenopus* oocytes expressing Gr10, Gr6, and both of them. Finally, we individually knocked out the two GR genes and obtained two homozygous mutants of *H. armigera*

by CRISPR/Cas9, and then detected changes in the electrophysiological and behavioral responses of the mutant larvae and adults to sugars. Taking all the results together, we demonstrate that larval and adult *H. armigera* used different GRs to detect sucrose in food.

Results

Electrophysiological responses of taste sensilla to sugars

We first investigated the electrophysiological responses of the lateral and medial sensilla styloconica in the larval maxillary galea to seven sugars in plants, including sucrose, fructose, and glucose. The lateral sensilla styloconica showed significant unicellular responses to 10 mM sucrose and fucose, with the firing rates of 169 ± 8.57 and 43 ± 9.78 spikes/s, respectively **(Figure 1A, Figure 1—figure supplement 1A).** The threshold concentrations for their responses to sucrose and fucose were 0.1 mM and 10 mM, respectively (**Figure 1B, Figure 1—figure supplement 1B**). The medial sensilla styloconica had a weak response to xylose (**Figure 1—figure supplement 1C**). We also compared the responses of the lateral sensilla styloconica to 0.05 mM sucrose, 5 mM fucose, and their mixture with the same concentrations, and found that the mixture induced higher responses with only one amplitude spikes and a firing rate of 71 ± 12.36 spikes/s, which was higher than the firing rates induced by 0.05 mM sucrose (39 ± 6.37 spikes/s) and 5 mM fucose (36 ± 8.87 spikes/s) (**Figure 1—figure supplement 1D**), confirming that the same cell responded to sucrose and fucose. We conclude that there is only one GSN sensitive to sucrose and fucose in each maxillary galea of larvae.

We also studied the responses of the taste sensilla in the tip of the antennae, foreleg tarsi, and proboscis of female adults to seven sugar compounds at 10 mM and the related dose responses (**Figure 1C–H**). The antennal sensilla responded to sucrose, fucose, and fructose positively (**Figure 1C**), the tarsal sensilla only responded to sucrose and fucose (**Figure 1E**), and the proboscis sensilla responded to sucrose, fucose, and fructose (**Figure 1G**), but their firing rates were between 9-22 spikes/s. All the evoked spike traces had one amplitude, indicating that they were single cell responses (**Figure 1—figure supplement 1A**). The dose–response curves showed that the threshold concentrations of sucrose, fucose and fructose were all 10 mM for the antennal sensilla (**Figure 1D, Figure 1—figure supplement 1B**), 100 mM, 10 mM and 100 mM for the tarsal sensilla (**Figure 1F**, **Figure 1—figure supplement 1B**), and 10 mM, 100 mM and 10 mM for the proboscis sensilla, respectively (**Figure 1H**, **Figure 1—figure supplement 1B**).

In short, both larvae and adults of *H. armigera* had sugar GSNs, but their response profiles, intensity and sensitivity differed distinctively. The sugar GSNs in lateral sensilla styloconica of larvae responded strongly to sucrose with high sensitivity, and weakly to fucose; while the sugar GSNs of adults responded to sucrose, fucose, and fructose (except for those on tarsi) with lower intensity and sensitivity. The GSNs of larvae were 100–1000 times more sensitive to sucrose than those of adults.

Behavioral responses of larvae and adults to sugars

The two-choice test was used to examine the feeding behavior of the 5th instar larvae of *H. armigera* to the seven sugars at 10 mM. Sucrose and fructose stimulated larval feeding, and their threshold concentrations were 10 mM (**Figure 2A–B, Figure**

2—figure supplement 1); other tested sugars had no effect on larval feeding (**Figure 2A**).

We also studied the proboscis extension reflex (PER) induced by sugar stimulation in the antennal and tarsal tip sensilla of virgin female adults. In consideration of the sugar content in nectar and the response intensity of the taste sensilla in adults, the test concentration of sugars was set at 100 mM. The results showed that the PER percentage reached 64 ± 4.35%, 44 ± 5.96% and 20 ± 1.92% when the antennal sensilla were stimulated with sucrose, fucose and fructose (**Figure 2C**), while the PER percentage reached 53 ± 14.08%, 45 ± 4.44% and 36 ± 10.19% when the tarsal sensilla were stimulated, respectively (**Figure 2D**). Stimulation of the antennal or tarsal sensilla by other sugar compounds did not induce changes in the PER percentage compared with stimulation of water (**Figure 2C and 2D**). The dose–response curves showed that the threshold concentrations of sucrose, fucose, and fructose for the antennal sensilla were 100 mM, 100 mM, and 1000 mM (**Figure 2E**), those for the tarsal sensilla were 10 mM, 10 mM, and 100 mM, respectively (**Figure 2F**).

Phylogenetic tree and expression patterns of putative sugar gustatory receptors

Based on the reported gene sequences of GRs in *H. armigera* (Jiang et al., 2015; Pearce et al., 2017; Xu et al., 2017), we cloned the full-length cDNA sequences of all nine putative sugar GR genes, *Gr4–12* (The GenBank accession number is provided in **Appendix—Table S1**). The lengths of the amino acid (aa) sequences of the sugar GRs were consistent with the lengths of the corresponding reported sequences (the similarity was 97%-99%), except for Gr8, Gr9, and Gr10 (Xu et al., 2017). Gr8 had

two more amino acids at the C-terminal than the reported by Xu et al., 2017 with 99% sequence similarity (Xu et al., 2017). Gr9 had 9 more amino acids at the N-terminal than the reported in the *H. armigera* genome study with 99% sequence similarity (Pearce et al., 2017), while the sequence length of Gr9 was exactly the same as Gr4 in Jiang et al., 2015 with 99% sequence similarity (Jiang et al., 2015). Gr10 had 20 more amino acids than the sequence reported in the *H. armigera* genome study (Pearce et al., 2017) and 186 more amino acids than the sequence reported by Xu et al., 2017 (Xu et al., 2017), with 97% and 99% sequence similarities, respectively.

We established a phylogenetic tree using sugar GR genes of nine insect species, including *D. melanogaster*, *Aedes aegypti*, and *Anopheles gambiae* in Diptera; *Apis mellifera* in Hymenoptera; and *Danaus plexippus*, *Heliconius melpomene*, *Bombyx mori*, *Plutella xylostella*, and *H. armigera* in Lepidoptera (**Figure 3A**). The phylogenetic analysis showed that candidate sugar GRs of these nine insect species grouped within two clades, the sugar GR clade and the fructose GR clade. All the putative sugar GRs of *H. armigera* were in the sugar GR clade except for HarmGr9, which was in the fructose GR clade (**Figure 3A**).

To determine the expression patterns of nine putative sugar GR genes in larval and adult taste organs, we measured their expression levels in the larval maxillary galea, and the antennae, foreleg tarsi, and proboscis in virgin female and male adults by real-time quantitative PCR (qRT-PCR) (**Figure 3B–3E, Figure 3—figure supplement 1**). The results showed that the expression patterns of sugar GRs in the same organ of male and female adults were nearly the same (**Figure 3C–3E**, **Figure 3—figure supplement 1**). The average expression level of all sugar GRs in the larval maxillary galea was lower than that in the adult organs. In each taste organ, the

expression levels of 1–2 sugar GRs were much higher than those of other sugar GRs, and these highly expressed GRs were significantly different between larvae and adults (**Figure 3B–3E, Figure 3—figure supplement 1**). Among all sugar GRs, *Gr10* was highly expressed in the larval maxillary galea, whereas *Gr6* was highly expressed in the antennae, tarsi, and proboscis of both male and female adults. *Gr5* expression levels greatly varied; they were highest in the proboscis followed by the tarsi, but very low in the antennae and the maxillary galea of the larvae (**Figure 3B–3E**, **Figure 3—figure supplement 1**).

Functional analysis of putative sugar gustatory receptors by ectopic expression

To clarify the functions of putative sugar GRs in *H. armigera* except for *Gr9*, which was named as *Gr4* in Jiang et al., 2015 and identified as a fructose receptor (Jiang et al., 2015), we first investigated the inward current changes in *Xenopus* oocytes expressing sugar GRs individually in response to the stimulation of 11 sugar compounds at 100 mM using two-electrode voltage-clamp. The results showed that the oocytes expressing Gr10 only responded to sucrose with a current value of 4.10 ± 0.53 μA (**Figure 4A–4B**); the oocytes expressing Gr6 showed responses to fucose and sucrose with a current value of 1.59 ± 0.08 μA and 0.24 ± 0.03 μA, respectively (**Figure 4A–4B**). The oocytes expressing remaining six sugar GR genes (Gr4, Gr5, Gr7, Gr8, Gr11, and Gr12) singly had no response to the tested sugar compounds (**Figure 4—figure supplement 1A–B**). The dose–response curves showed that the threshold concentration of sucrose for the oocytes expressing Gr10 was 100 mM (**Figure 4C–4D**); whereas the threshold concentrations of sucrose and fucose for the oocytes expressing Gr6 were 250 mM and 100 mM, respectively, and 500 mM fructose could also induce an inward current (**Figure 4C–4D**).

203 Given that there was only one sugar GSN responding to sucrose and fucose in each
204 larval maxillary galea, we speculated that Gr10 should be expressed together with Gr6
205 in this cell although their expressing levels were different. Therefore, we examined
206 the response profile of the oocytes expressing Gr10 together with Gr6. The results
207 showed that the oocytes co-expressing Gr10 and Gr6 significantly responded to
208 sucrose with a current value of 1.89 ± 0.58 μA at 100 mM, a threshold concentration,
209 and had a weak response to fucose with a threshold concentration of 250 mM (**Figure**
210 **4**).

211 Since Gr5 was highly expressed with Gr6 in the proboscis and tarsi (**Figure 3D-3E,**
212 **Figure 3—figure supplement 1**), we suspected that Gr5 could be a partner of Gr6
213 and then tested the response profile of their co-expression in oocytes. Similar to the
214 oocytes expressing Gr6, the oocytes expressing Gr5 and Gr6 had responses to 100
215 mM fucose with a current value of 0.11 ± 0.015 μA and to 100 mM sucrose with a
216 current value of 0.03 ± 0.002 μA (**Figure 4—figure supplement 1B–C**).

217 In summary, we find that oocytes expressing single Gr10 or Gr6 confer sensitivity to
218 one or more sugars, indicating their direct roles in ligand recognition. Gr10 is tuned to
219 sucrose specifically, Gr6 is responsive to fucose, sucrose and fructose in the order of
220 sensitivity, and the combination of Gr10 and Gr6 is sensitive to sucrose and then
221 fucose, which is in line with the characteristics of the sugar GSNs in larval and adult
222 taste organs where the two GRs are highly expressed respectively.

223 **Establishment of *Gr10* and *Gr6* homozygous mutants**

To validate the function of *Gr10* and *Gr6 in vivo*, we further constructed *Gr10* and *Gr6* homozygous mutants (*Gr10*$^{-/-}$ and *Gr6*$^{-/-}$, respectively) using CRISPR/Cas9 techniques (**Figure 5**). *Gr10*$^{-/-}$ and *Gr6*$^{-/-}$ generated 4-bp and 17-bp deletions at the sgRNA targeting site **(Figure 5)**, respectively, which also confirmed the deletion at the mRNA level in *Gr10*$^{-/-}$ and *Gr6*$^{-/-}$ **(Figure 5—figure supplement 1)**. The deletion resulted in truncated Gr10 and Gr6 proteins. The Gr10 sequence was reduced from 460 to 62 amino acids and contained no transmembrane domains, whereas the Gr6 sequence was reduced from 452 to 115 amino acids and contained only two transmembrane domains **(Figure 5)**.

According to the predicted results from Cas-OFFinder, the *Gr10* sgRNA had no potential off-target region but *Gr6* sgRNA had one. Therefore, we amplified and sequenced the potential off-target region of *Gr6*$^{-/-}$ and found there was no frameshift or premature stop codon in the region compared to WT **(Figure 5—figure supplement 2)**. We also found there was no difference in the response to xylose of the medial sensilla styloconica among WT, *Gr10*$^{-/-}$ and *Gr6*$^{-/-}$ **(Figure 5—figure supplement 2**). Furthermore, WT, *Gr10*$^{-/-}$ and *Gr6*$^{-/-}$did not show differences in the larval body weight, adult lifespan, and number of eggs laid per female (**Figure 5—figure supplement 2**). All these results suggest that no off-target effects occurred in the study.

Effects of *Gr10* or *Gr6* knockout on sugar reception

We measured electrophysiological responses of the sugar GSNs in larval lateral sensilla styloconica of the WT, *Gr10*$^{-/-}$, and *Gr6*$^{-/-}$ to sucrose and fucose using 10 mM sinigrin as a positive control, which induced the response of a bitter cell in the same

247 sensilla. To 1 mM, 10 mM and 100 mM sucrose, the firing rates of the larval sugar
248 GSNs in *Gr10*$^{-/-}$ were only 40%, 20% and 28% of those in the WT, respectively,
249 whereas those in *Gr6*$^{-/-}$ larvae did not differ from those in the WT larvae (**Figure 6A–**
250 **6B**). To 10 mM and 100 mM fucose, the firing rates of the larval sugar GSNs in *Gr6*$^{-/-}$
251 were only 20% and 5.8% of those in the WT, respectively, whereas those of *Gr10*$^{-/-}$
252 did not differ from the WT (**Figure 6A–6B**). The electrophysiological responses of
253 the bitter GSNs to sinigrin in the larval lateral sensilla styloconica of the WT, *Gr10*$^{-/-}$,
254 and *Gr6*$^{-/-}$ were all strong and did not differ (**Figure 6A–6B**). It is clear that *Gr10*
255 plays a key role in sucrose reception whereas *Gr6* plays a key role in fucose reception
256 of the sugar GSNs in the lateral sensilla styloconica of larvae.

257 Similarly, we measured the electrophysiological responses of the sugar GSNs in taste
258 sensilla of the adult antennae, foreleg tarsi, and proboscis of the WT, *Gr10*$^{-/-}$, and
259 *Gr6*$^{-/-}$ to 10 mM and 100 mM of sucrose and fucose, and 100 mM fructose.
260 Additionally, 10 mM nicotine was used as a positive control, that induced
261 electrophysiological responses of bitter GSNs in the tip sensilla of antennae and tarsi.
262 The firing rates of the sugar GSNs in the antennal sensilla of *Gr6*$^{-/-}$ to 10 mM and 100
263 mM sucrose, 10 mM and 100 mM fucose, and 100 mM fructose were only 32% and
264 9%, 22% and 11%, and 29% of those in the WT, respectively (**Figure 6C–6D**). The
265 firing rates of the sugar GSNs in antennal sensilla of *Gr10*$^{-/-}$ to sucrose, fucose, or
266 fructose did not differ from those of the WT adults. The antennal sensilla of the WT,
267 *Gr10*$^{-/-}$, and *Gr6*$^{-/-}$ all showed strong responses to nicotine with no differences (**Figure**
268 **6C–6D**). The response patterns of the sugar GSNs in the foreleg tarsi of the WT,
269 *Gr10*$^{-/-}$, and *Gr6*$^{-/-}$ to 10 mM and 100 mM sucrose, 10 mM and 100 mM fucose, and
270 100 mM fructose were similar to those of the sugar GSNs in antennae (**Figure 6E–**

271 **6F**). The firing rates of the sugar GSNs in proboscis sensilla of *Gr6*$^{-/-}$ were much
272 lower and even close to zero (**Figure 6G–6H**). The firing rates of the sugar GSNs in
273 tarsal and proboscis sensilla of *Gr10*$^{-/-}$ to sucrose, fucose, or fructose did not differ
274 from those of the WT adults (**Figure 6E–6H**). The tarsal sensilla of the WT, *Gr10*$^{-/-}$,
275 and *Gr6*$^{-/-}$ responded to nicotine with no difference (**Figure 6E–6F**).

276 **Effects of *Gr10* and *Gr6* knockout on behavioral responses**

277 To determine the effect of *Gr10* and *Gr6* knockout on feeding responses of *H.*
278 *armigera* larvae, we compared the larval feeding preferences of the WT, *Gr10*$^{-/-}$, and
279 *Gr6*$^{-/-}$ to sucrose by two-choice tests. The *Gr10*$^{-/-}$ larvae showed no preference for the
280 leaf discs painted with 10 mM sucrose (P = 0.4258). However, they still had
281 preference for the discs with 100 mM sucrose (P = 0.0141) (**Figure 7A–7B**) though
282 their PI value was decreased than those of the WT (**Figure 7B**). The *Gr6*$^{-/-}$ larvae
283 were the same as the WT larvae and retained a significant preference for 10 mM or
284 100 mM sucrose diets (**Figure 7A–7B**). The WT, *Gr10*$^{-/-}$, and *Gr6*$^{-/-}$ larvae all showed
285 similar preference for 10 mM and 100 mM fructose **(Figure 7—figure supplement**
286 **1A–B)**.

287 We also compared the feeding amount in 1 hour of the larvae of WT, *Gr10*$^{-/-}$, and
288 *Gr6*$^{-/-}$ on cabbage leaves, corn kernels, pea seeds, pepper fruits, and tomato fruits by
289 no-choice tests. On each of these plant tissues, the *Gr10*$^{-/-}$ larvae fed significantly less
290 than the WT larvae, but the *Gr6*$^{-/-}$ larvae fed the same amount as the WT larvae
291 (**Figure 7C**).

292 Moreover, we examined the PER of the WT, *Gr10*$^{-/-}$, and *Gr6*$^{-/-}$ female adult antennae
293 and tarsi in response to stimulation of sucrose, fucose, and fructose at different

concentrations. When antennae or tarsi were stimulated with 10 mM, 100 mM, or 1000 mM sucrose, fucose, or fructose, *Gr6*$^{-/-}$ females showed a considerably lower PER percentage than the WT females and even had little or no PER in response to sucrose and fucose, whereas the PER percentage of *Gr10*$^{-/-}$ females was not different from that of the WT females (**Figure 7D–7I**).

In conclusion, *Gr10* plays a key role in sucrose reception by the sugar GSNs in the larval lateral sensilla styloconica, and mediates the feeding preference of larvae to sucrose, whereas *Gr6* plays a key role in sensing sucrose, fucose, and fructose by the sugar GSNs in taste sensilla of the adult antennae, foreleg tarsi, and proboscis, and mediates the related PER behavior of adults.

Discussion

In this study, we reveal the different molecular bases of sucrose reception between *H. armigera* larvae and adults through ectopic expression and genetic editing of GRs. *Gr10*, which was highly expressed in the larval maxillary galea, is specifically tuned to sucrose with high sensitivity, whereas *Gr6*, which was highly expressed in the adult antennae and foreleg tarsi, has a broader response spectrum but lower sensitivity to sucrose, indicating that larvae mainly use *Gr10* to detect low sucrose in plant tissues, while adults primarily use *Gr6* to detect a variety of sugars with high content, including sucrose in nectar.

Our findings indicate that the *H. armigera* larvae and adults adopt different sugar-sensing systems to satisfy their requirements for food selection. The larvae

316 mainly feed on young leaves, flower buds, and fruits of host plants. As a universal
317 phagostimulant, sucrose is the major sugar in plants and considerably varies in
318 content in different tissues and developmental stages. Tomato is a principal host plant
319 of *H. armigera*. The sucrose content in its leaves generally ranges from 13.72 to 42.06
320 mM, but that in its fruits generally ranges from 35.2 to 988.22 mM (Schauer et al.,
321 2005). The adults mainly feed on plant nectar. Sucrose and some monosaccharides,
322 especially fructose and glucose, are the main components in nectar, where the sugar
323 content is much higher than that in leaves and fruits. The sugar content in the nectar
324 of cotton plants is 10%–34% (which roughly corresponds to 0.5 M–1.7 M) (Vansell,
325 1944; Waller et al., 1981), and that of soybean plants is even higher, around 23%–60%
326 (which roughly corresponds to 1.2–3 M) (Free, 1993). It is clear that, in their life
327 cycle, a single sugar-sensing mechanism cannot meet the requirements for food
328 selection by this phytophagous insect species. From the perspective of insect and
329 plant co-evolution, using value-based and target-specific sugar-sensing systems is a
330 favorable choice for phytophagous insects to ensure their growth and reproduction on
331 plants.

332 Although *Gr10* plays a major role in sucrose reception in the larval stage, the
333 response of the sugar GSNs in larval lateral sensilla styloconica to sucrose did not
334 completely disappear in *Gr10*$^{-/-}$ (**Figure 6A–6B**), and the larvae still showed a
335 behavioral preference for the high concentration of sucrose (100 mM) (**Figure 7B**). It
336 is most likely that low-level expressed *Gr6* is functional in the GSNs. The PER
337 percentage of *Gr6*$^{-/-}$ adults was not disappeared when the antennae and tarsi were
338 stimulated by high fructose concentrations (**Figure 7F and 7I**); this indicates that
339 fructose-tuned GRs other than *Gr6* were involved. Previous studies have shown that

340 *Gr9* (*Gr4* in Jiang et al., 2015), as an ortholog of DmelGr43a and BmorGr9, is a
341 fructose-tuned GR (Ai et al., 2022; Jiang et al., 2015; Sato et al., 2011; Xu et al.,
342 2012). A thorough understanding of the sugar coding mechanisms will require
343 comparison of a series of single, double and even multiple knock-out lines.

344 The larval maxillary galea with only two sensilla styloconica is an ideal system for
345 studying the mechanisms of sugar sensing. In each maxillary galea of *H. armigera*,
346 the only sugar GSN sensitive to sucrose and fucose is located in the lateral sensillum
347 styloconicum. Based on our results, we speculate that Gr10 and Gr6 are expressed in
348 this GSN with different levels, and mainly determine the response profile of the cell.
349 The firing rate of the cell in *Gr10*$^{-/-}$ to 1mM, 10 mM and 100 mM sucrose was
350 significantly lower than that in WT, but there was no difference between WT and
351 *Gr6*$^{-/-}$, indicating that Gr10 mainly determine the sensitivity of this cell to sucrose
352 (**Figure 6A–6B**). In contrast, the firing rate of the cell in *Gr6*$^{-/-}$ to 10 mM and 100
353 mM fucose was significantly lower than that in the WT, but there was no difference
354 between WT and *Gr10*$^{-/-}$, indicating that Gr6 was also functional in larvae (**Figure**
355 **6A–6B**). This can be well explained by the response profiles of the oocytes expressing
356 Gr10, Gr6, and their combination **(Figure 4)**.

357 Sugars are common phagostimulants in animals, but the molecular basis of sugar
358 perception differs among animals. In mammals, the taste receptors T1R2 and T1R3
359 combine to complete the perception of all carbohydrate compounds, including sucrose,
360 and both receptors are indispensable (Damak et al., 2003; Li et al., 2002; Nelson et al.,
361 2001; Zhao et al., 2003). In *D. melanogaster* adults, multiple sugar GRs are involved
362 in sugar reception, different GRs expressed in sweet taste neurons may function as
363 multimers (Dahanukar et al., 2001; Dahanukar et al., 2007; Fujii et al., 2015; Jiao et

364 al., 2007; Jiao et al., 2008; Slone et al., 2007). Gr5a and Gr64a are widely used for
365 responses to complementary subsets of sugars in labellar sweet taste neurons
366 (Dahanukar et al., 2007), and Gr64f is also required broadly as a coreceptor for the
367 detection of sugars (Jiao et al., 2008). The recent successful ectopic expression of
368 single sugar GRs in olfactory neurons confirmed that each sugar GR is directly
369 involved in ligand recognition (Freeman et al., 2014). Sweet sensing neurons or
370 receptors have not been identified in the larval peripheral taste system (Kwon et al.,
371 2011). Sugar perception in *Drosophila* larvae has been assigned to the fructose
372 receptor *Gr43a*, which, however, is expressed in pharyngeal sensory organs, foregut,
373 and in the brain, but absent from external taste neurons (Mishra et al., 2013). Anyway,
374 the sucrose sensing neurons were recently identified in the larval primary taste center
375 (Maier et al., 2021). In honeybees, AmGr1 acts as a GR for certain sugars other than
376 fructose, and AmGr2 acts as a coreceptor with AmGr1 (Jung et al., 2015). AmGr3 is
377 tuned to fructose and is phylogenetically closely related to the DmGr43a (Değirmenci
378 et al., 2020; Takada et al., 2018). The whitefly *Bemisia tabaci* consistently choose
379 diets containing higher sucrose concentrations. It has been shown that BtabGr1
380 displayed significant sucrose specificity when expressed in *Xenopus* oocytes, and
381 silencing of this GR significantly interfered with the ability of *B. tabaci* adults to
382 detect sucrose in the phloem sap (Harari et al., 2023).

383 The most studied phytophagous insects for sugar perception are Lepidopterans.
384 BmGr9 in *B. mori* (Sato et al., 2011) (Morinaga et al., 2022), HarmGr9 in *H.*
385 *armigera* (named as HarmGr4 by Jiang et al., 2015) (Jiang et al., 2015; Xu et al.,
386 2012), PxGr43a-1 in *P. xylostella* (Liu et al., 2020), and SlGr8 in *Spodoptera litura*
387 (Liu et al., 2019) were all the orthologs of DmGr43a in *D. melanogaster* (Miyamoto

et al., 2012), and tuned to fructose without complexing with other GRs. BmGr4 was recently identified as a receptor for sucrose and glucose and may function as a chemosensor for nutritional compounds in both midgut and brain (Mang et al., 2022). In this study, we find *Gr10* and *Gr6* were highly expressed in the larval and adult taste organs, respectively. The experiments on ectopic expression in *Xenopus* oocytes shows that Gr10 specifically responded to sucrose, Gr6 responded to fucose and sucrose, and weakly to fructose, and the combination of Gr10 and Gr6 responded to sucrose and weakly to fucose (**Figure 4A–4B**). These results indicate that Gr10 and Gr6 are capable of forming functional receptors singly, and the function of the two co-expressed receptors is not equal to the sum of the functions of the two receptors **(Figure 4)**. When further tracing the genomic locations of the two GR genes, we confirm that *Gr10* and *Gr6* together with other 6 sugar GR genes (except for *Gr9*) are tandemly duplicated on the same scaffold of *H. armigera* (Pearce et al., 2017). The sugar GR gene cluster and interactions between GRs could provide a degree of evolutionary and regulatory flexibility that may meet the needs of the insect to sense subtle sugar components and concentrations in food sources in a changing environment (Delventhal & Carlson, 2016).

In this work, we report for the first time the molecular basis of sucrose reception in external taste neurons of a Lepidopteran insect, and discover that different taste receptors are used for food selection in *H. armigera* larval and adult stages. These findings greatly improve our understanding of “sweet” taste of phytophagous insects. According to the phylogenetic tree of insect sugar GRs, *Gr10* is clustered in the same branch with BmorGr5 of *Bombyx mori*, whereas *Gr6* is clustered in another branch with BmorGr6 (**Figure 3A**). GRs closely associated with *Gr10* and *Gr6* are also

found in other Lepidopteran species such as *D. plexippus*, *H. melpomene*, and *P. xylostella* (**Figure 3A**). Therefore, we speculate that similar sugar-sensing mechanisms may also exist in other Lepidopteran species, which is worth verifying in these species in the future. To thoroughly reveal the mechanisms of sugar sensing by phytophagous insects and provide a new approach for pest control, it is necessary to study the function of a complete set of sugar receptors.

Materials and Methods

Insects

Helicoverpa armigera were collected from Xuchang (Henan Province, China) and raised in an indoor environment (27±1°C, 70%–80% humidity) with a 16 : 8 h light : dark cycle. The larvae fed on artificial diet (mainly including wheat bran, wheat germ, and tomato paste) in a glass tube (I.D., 2.5 cm; length, 8 cm). When the larvae reached the 3rd instar, they were transferred to a new glass tube and raised individually until pupation. Female and male moths were raised separately until they became sexually mature and then moved to a cage for mating. Since the taste system of the female is similar to that of the male and the nutrient requirements are more critical for females (Jiang et al., 2015; Zhang et al., 2010), the virgin female adults were used in the related experiments.

Xenopus laevis

Xenopus laevis were fostered at 20 ± 1°C in the Animal Laboratory Center, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences. They fed on artificial frog food (mainly including fish meal, yeast extract powder, high-gluten flour). All experiments were approved by the Animal Care and Use Committee of the Institute of Zoology, Chinese Academy of Sciences and followed the Guide for the Care and Use of Laboratory Animals (IOZ17090-A).

Tip-recording

The tip-recording technique based on a previously described methods (Hodgson et al., 1955; van Loon, 1990; Zhang et al., 2010) was used to record the electrophysiological responses of lateral and medial sensilla styloconica in larval maxillary galea, sensilla chaetica in adult antennae, sensilla trichodea in adult tarsi, and sensilla styloconica in adult proboscis of *H. armigera*. A glass capillary filled with the test compound solution, into which a silver wire was inserted, was placed in contact with the taste sensillum to be recorded as the recording electrode. The tip diameter of the electrode was about 50 μm, suitable for recording from a single sensillum. Action potentials (spikes) generated during the first second after stimulus onset were amplified by a preamplifier and was sampled with a computer equipped with a Metrabyte DAS16 A/D conversion board. The amplifier used an AD 515-K (Analog Devices) integrated circuit in the first stage, yielding < 1 pA input bias current, 1015 Ohm and 0.8 pF input impedance. An interface (GO-box) was used for signal conditioning. This involved a second-order band pass filter (-3 dB frequencies: 180 and 1700 Hz). Stimuli were dissolved in 2 mM KCl, which was used as the control. Arabinose, fructose, fucose, galactose, glucose, sucrose, and xylose (**Key resources table**) were first tested at 10 mM to screen active compounds, which were then tested in a range

456 of concentrations in dose-response assays (0.01 mM, 0.1 mM, 1 mM, 10 mM and 100
457 mM). To avoid possible adaptation of the taste sensilla tested, the interval between
458 two successive stimulations was at least 3 min. Digitized traces were analyzed by
459 means of SAPID Tools software version 3.5 (Smith et al., 1990) and Autospike 3.7
460 (Syntech, Buchenbach, Germany). The spikes were sorted on the basis of typical
461 biphasic waveforms and spike amplitudes, and the frequency of the same type of
462 spikes was counted from the first second after stimulation.

463 In recording of electrophysiological responses of larvae, the first day of larvae in the
464 5th instar were used. To guarantee all test caterpillars were in the same stage, we first
465 selected 4th instar larvae which were preparing to undergo ecdysis, and then
466 transferred them to a glass tube with green pepper. The test larva was starved for
467 approximately 2 h before recording. The head was cut and fixed in a silver holder
468 which was connected to a preamplifier with a copper miniconnector. The lateral and
469 medial sensilla styloconica in larval maxillary galea were exposed for testing
470 electrophysiological responses. About 12–13 replicates were set for each treatment.

471 The virgin female moths on the second day after eclosion were used for recording
472 electrophysiological responses (Jiang et al., 2015; Zhang et al., 2010). They were only
473 fed with ddH_2O. When testing sensilla in a tarsus, the foreleg was cut between coxa
474 and femur, and the silver holder connected to the amplifier was inserted into the femur.
475 When testing sensilla in the antennae and proboscis, the head and thorax without all
476 legs were reserved. The silver holder was inserted into the thorax. The orientation of
477 the taste organ was gently adjusted to allow the sensilla to be touched by the electrode.
478 To guarantee the sensilla was steadily exposed, the distal part of antenna or proboscis
479 was fixed on a small platform with double sided adhesive tape. For each individual,

three sensilla on the top area of antennae, foreleg tarsi, or proboscis were chosen for testing electrophysiological responses, and about 15–21 replicates were used for each treatment.

Two-choice tests

We determined the behavioral responses to sugars of the *H. armigera* larvae using the two-choice test as previously described with small modifications (Chen et al., 2022). We used the leaf of cabbage (*Brassica oleraceaas*, Shenglv-7) as the substrate since it is not a favorite food for larvae. Circular cabbage leaf discs of 1 cm in diameter were prepared. The upper surface of the treated leaf disc was painted with the 35.2 μL sugar solution with 0.01% Tween 80 (CAS: 9005-55-6, Sigma-Aldrich, St Louis, USA) using a paintbrush, and the control leaf disc was painted with 35.2 μL ddH_2O with 0.01% Tween 80. The feeding preference of larvae to seven sugars including arabinose, fructose, fucose, galactose, glucose, sucrose, and xylose (**Key resources table**) was tested at 10 mM. 0.01 mM, 0.1 mM, 1 mM, 10 mM, and 100 mM sucrose and fructose were used in a dose-response assay. The preference index (PI) was calculated according to the following formula: PI = (the feeding area of test leaf discs – the feeding area of control leaf discs) / (the feeding area of test leaf discs + the feeding area of control leaf discs). 20 replicates were run.

Adult proboscis extension reflex (PER) tests

We determined the behavioral responses to sugars of the *H. armigera* female adults using the proboscis extension reflex (PER) test as previously described with some modifications (Jiang et al., 2015). Arabinose, fructose, fucose, galactose, glucose, sucrose, and xylose (**Key resources table**) were first tested at 100 mM to screen

503 active compounds, which were then tested in a range of concentrations in
504 dose-response assays (0.01 mM, 0.1 mM, 1 mM, 10 mM, 100 mM, and 1000 mM). In
505 brief, the virgin females on the first day after eclosion were used. Each moth was
506 harnessed in a 1 mL pipette with a tip removed, from which its head protruded, and its
507 antennae, proboscis and forelegs were permitted to move freely. They were allowed to
508 adapt to the environment for about 12 h, and satiated with ddH_2O until the trial. The
509 testing moth was stimulated to the distal part of one antenna with a given test solution
510 in an absorbent cotton ball, and the PER in response to the stimulation was recorded.
511 The interval between two successive stimulations was 5 min, during which the distal
512 part of the antenna was washed, and the insect was fed with ddH_2O if needed. Four
513 hours later, two foreleg tarsi of the moth were simultaneously stimulated with the test
514 solutions in the same way as the antenna was stimulated, and the PER was recorded.
515 20 to 30 individual moths were used for each set of tests. The experiments were
516 repeated three times with groups of insects from three different rearing batches.

517 **RNA extraction and cDNA synthesis**

518 The galea of larvae, and the antennae, foreleg tarsi, proboscis, and abdomen of both
519 male and female adults were removed and directly placed into 1.5 mL Eppendorf
520 tubes (Axygen, Tewksbury, MA, USA) containing 1 mL QIAzol Lysis Reagent
521 (QIAGEN, Hilden, Germany); then, the extraction of total RNA was carried out
522 following the manufacturer's protocols. After extraction, the RNA quality and
523 concentration were determined with a NanoDrop 2000 spectrophotometer (Thermo
524 Fisher Scientific, Waltham, MA, USA). First, for cDNA synthesis 2 μg total RNA
525 was mixed with 1 μL oligo-dT (Promega, Madison, WI, USA); then, the mixture was
526 heated at 70°C 5 min and followed by an ice bath for 5 min. M-MLV reverse

527 transcriptase (Promega, Madison, WI, USA) was added; subsequently, the mixture
528 was incubated at 42°C for 90 min. The products were dissolved in ddH_2O and stored
529 at −20°C for later use.

530 **Gene cloning of sugar GRs**

531 We designed specific primers (**Appendix—Table 2**) according to the reported sugar
532 GR sequence of *H. armigera* (the GenBank accession number is provided in
533 **Appendix—Table 1**) for PCR cloning (Pearce et al., 2017; Xu et al., 2017). The
534 GenBank accession number of *Gr9* (named as *Gr4* in Jiang et al., 2015) nucleic acid
535 sequence and protein sequence were JX982536.2 and AKG90011 in Jiang et al., 2015,
536 respectively (Jiang et al., 2015). The Q5 High-fidelity DNA Polymerase (New
537 England Biolabs, Beverly, MA, USA) was used to clone sugar sequences except *Gr10*.
538 The PCR instrument was Applied Biosystems (Thermo Fisher Scientific, Woodlands,
539 Singapore) and the sequence amplification procedure was as follows: 98°C for 10 s,
540 40 cycles (60°C for 30 s, 72°C for 1 min), and final extension at 72°C for 2 min.
541 TransStart FastPfu DNA Polymerase (TransGen Biotech, Beijing, China) was used to
542 clone the *Gr10* sequence. The amplification procedure was as follows: 95°C for 1 min,
543 40 cycles (95°C for 20 s, 50°C for 20 s, 72°C for 45 s), and 72°C for 5 min. PCR
544 products were purified, subcloned into pGEM-T vector according to the
545 manufacturer's instructions (Promega), and sequenced. *Gr4* (402 amino acids) and
546 *Gr7* (428 amino acids) were obtained from the tissues of male and female *H.*
547 *armigera* tarsi, respectively. *Gr5* (482 amino acids), *Gr6* (452 amino acids), and *Gr10*
548 (460 amino acids) were all obtained from the tissues of female *H. armigera* proboscis.
549 *Gr8* (427 amino acids) and *Gr9* (474 amino acids) was obtained from the tissues of
550 female *H. armigera* antennae. Finally, *Gr11* (435 amino acids) and *Gr12* (434 amino

551 acids) were obtained from the tissues of female and male *H. armigera* abdomens,
552 respectively. No alternatively spliced transcript variants were found in the gene
553 cloning of all the sugar GRs studied.

554 Xu et al., 2017 (Xu et al., 2017) reported that *Gr10* was a pseudogene because the
555 nucleotide sequence changed at positions 521–524 compared with the sequence
556 reported in Pearce et al., 2017 (Pearce et al., 2017), and it terminated early during
557 translation. The *Gr10* sequence that we obtained had no termination on the same
558 position. Aligning the *Gr10* sequences of Pearce et al., 2017 and Xu et al., 2017
559 revealed that *Gr10* in Xu et al., 2017 had 22 more amino acids at the C-terminus than
560 that in Pearce et al., 2017. Therefore, we designed upstream and downstream primers
561 of *Gr10* from the N-terminus in Pearce et al., 2017 and the C-terminus in Xu et al.,
562 2017. Finally, we obtained the full-length sequence of *Gr10* with 460 amino acids.

563 **Phylogenetic analysis of sugar GRs**

564 The phylogenetic tree was built with *H. armigera* and sugar GRs of eight other insect
565 species: Diptera, *Drosophila melanogaster* (Robertson et al., 2003), *Aedes aegypti*
566 (Kent et al., 2008), and *Anopheles gambiae* (Hill et al., 2002); Hymenoptera, *Apis*
567 *mellifera* (Robertson & Wanner, 2006); and Lepidoptera, *Danaus plexippus* (Zhan et
568 al., 2011), *Heliconius Melpomene* (Briscoe et al., 2013), *Bombyx mori* (Guo et al.,
569 2017), and *Plutella xylostella* (Engsontia et al., 2014). Amino acid sequences were
570 aligned with MAFFT version 7.455 (Rozewicki et al., 2019), and gap sites were
571 removed with trimAI version 1.4 (Capella-Gutierrez et al., 2009). The phylogenetic
572 tree was constructed using the maximum likelihood method in IQ-tree version 6.8
573 (Nguyen et al., 2015). The bootstrap algorithm was based on the Jones–Taylor–

574 Thornton (JTT) + F + G4 model and run 5000 times. Visualization and later
575 modification of phylogenetic trees were performed in FigTree version 1.4.4
576 (http://tree.bio.ed.ac.uk/software/figtree/).

577 **Quantitative real-time PCR**

578 The relative expression levels of sugar GRs in *H. armigera* were analyzed by
579 quantitative real-time PCR (qRT-PCR) (Livak & Schmittgen, 2001). The specific
580 primers **(Appendix—Table 3**) were designed by Primer3plus
581 (http://www.primer3plus.com/cgi-bin/dev/primer3plus.cgi). qRT-PCR was conducted
582 with a QuantStudio 3 Real-Time PCR System (Thermo Fisher Scientific, Waltham,
583 MA, USA). All reactions included 10 μL SYBR Premix Ex *Taq* (TaKaRa, Shiga,
584 Japan); 0.4 μL (10 mM) primer (forward and reverse), 2 μL template DNA, 0.4 μL
585 ROX Reference Dye II Dye, 6.8 μL ddH_2O (20 μL total). The procedure was as
586 follows: 95°C for 30 s; 40 cycles of 95°C for 5 s and 60°C for 34 s; 95°C for 15 s;
587 60°C for 1 min; and 95°C for 15 s. *18S* ribosomal RNA (GenBank number:
588 KT343378.1) was used as the standard gene because the Ct value of this gene was not
589 different between different tissues (Sharath Chandra et al., 2014). The relative
590 expression levels of the specific genes were quantified by using the $2^{-\Delta Ct}$ method,
591 where ΔCt is the Ct value of GR genes subtracted from that of *18S*. Three biological
592 replicates were run for all experiments, and there were 30–100 insects in each
593 replicate.

594 **Ectopic expression and functional analysis of sugar GRs**

595 The sugar GRs sequences which were used for ectopic expression and functional
596 identification need to contain restriction enzyme sites and Kozak sequences (the
597 primer sequences of *H. armigera* sugar GRs for functional identification are provided
598 in **Appendix—Table 4**). The full-length sequence of the sugar receptor was cloned
599 from cDNA and transferred into pGEM-T vector (Promega, Madison, WI, USA).
600 After cleavage by appropriate endonucleases, it was linked to $pCS2^+$ vector. $pCS2^+$
601 vector was linearized and cRNA was synthesized according to the protocol of the
602 mMESSAGE mMACHINE SP6 Kit (Ambion, Austin, TX, USA). The products were
603 dissolved in ddH_2O, diluted to 3000 ng/μL, and then stored at −80°C until use.

604 Each of Gr4, Gr5, Gr6, Gr7, Gr8, Gr10, Gr11, Gr12, Gr5 + Gr6, and Gr10 + Gr6 was
605 expressed in oocytes. The acquisition of *X. laevis* oocytes was performed following a
606 previously described protocol (Nakagawa & Touhara, 2013). *X. laevis* were
607 anesthetized by a mixture of ice and water for 30 min; then, the abdomen was cut to
608 obtain oocytes. The *Xenopus* oocytes were treated with 2 mg/mL collagenase type I
609 (Sigma-Aldrich, St Louis, MO, USA) in a calcium-free saline buffer (82.5 mM NaCl,
610 2 mM KCl, 1 mM $MgCl_2$, 5 mM HEPES, pH = 7.5; the reagents are provided in **Key**
611 **resource table**) at room temperature for 25 min. The oocytes were microinjected with
612 27.6 nL (80 ng) cRNA of each *GR* by SMART Touch™ (World Precision
613 Instruments, Sarasota, FL, USA). For Gr10 + Gr6 or Gr5 + Gr6, a cRNA mixture with
614 a ratio of 1:1 was used. The oocytes microinjected with RNAse-free water were used
615 as the control. Then, the oocytes were incubated for 3–4 days at 16°C in the bath
616 solution (96 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$, 1.8 mM $CaCl_2$, 5 mM HEPES, pH
617 7.5; reagents are provided in **Key resource table**).

618 The whole-cell current was recorded with a two-electrode voltage clamp, the
619 procedures as previously described (Jiang et al., 2015). The glass electrode, filled with
620 3M KCl, was inserted into the oocytes and maintained at 0.2–2.0 MΩ. The signal was
621 amplified with an OC-725C amplifier (Warner Instruments, Hamden, CT, USA) at a
622 holding potential at –80 mV all the time, low-pass filtered at 50 Hz, and digitized at 1
623 kHz. The sugar compounds (**Key resource table**) were dissolved in 1 × Ringer
624 solution (96 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$, 1.8 mM $CaCl_2$, 5 mM HEPES, pH
625 7.5) as the test solution. The oocytes were stimulated by test solution via the perfusion
626 system for 20 s each time, and then washed by 1 × Ringer solution [68]. The
627 concentration of 100 mM for each chemical was randomly used at first, and then
628 concentration gradients (ranging from 0.01 mM, 0.1 mM, 1 mM, 10 mM, 50 mM, 100
629 mM, 250 mM, and 500 mM) were recorded later when a clear current response was
630 detected. Data were obtained and analyzed using Digidata 1322A and pCLAMP
631 software version 10.4.2.0 (Axon Instruments Inc., Foster City, CA, USA).

632 ***In vitro* synthesis of single-guide RNA**

633 *Gr10* and *Gr6* single-guide RNA (sgRNA) position screening was conducted at
634 http://www.rgenome.net/cas-designer/. The sgRNA target site of *Gr10* was designed
635 on exon 4 (**Figure 5B**), and the sgRNA target site of *Gr6* was designed on exon 2
636 (**Figure 5E**). The *Gr10* and *Gr6* sgRNA primers (**Appendix—Table 5**) were designed
637 based on the principles provided by the GeneArt™ Precision gRNA Synthesis Kit
638 manual (Invitrogen, Vilnius, Lithuania). The sgRNAs were synthesized by the
639 GeneArt™ gRNA Prep Kit (Invitrogen, Vilnius, Lithuania), and purified by the
640 GeneArt™ gRNA Clean Up Kit (Invitrogen, Vilnius, Lithuania). The products were
641 dissolved in RNAse-free water and then stored at −80°C until use. TrueCut™ Cas9

642 protein 2 (Invitrogen, Vilnius, Lithuania) was diluted to 500 ng/mL and stored at
643 −20°C.

644 **Embryo microinjection**

645 The fresh laid eggs of *H. armigera* were placed on microscope slides and fixed on
646 double-sided adhesive tape. These operations were finished within 2 h after
647 oviposition. Microinjection into these pretreated eggs was performed with a mixture
648 of *Gr10* (or *Gr6*) sgRNA and Cas9 protein, with final concentrations at 250 ng/□L
649 and 100 ng/μL, respectively. Microinjection was finished using a PLI-100A
650 PICO-INJECTOR (Warner Instruments, Hamden, Connecticut, USA). The injected
651 eggs were placed in a 10-cm Petri dish, covered with wet filter paper, and incubated at
652 25°C until hatching.

653 **DNA extraction and mutagenesis detection**

654 To screen *Gr10* (or *Gr6*) homozygous mutants, the tarsi on hindlegs were used for
655 DNA extraction with the Animal Tissue PCR Kit (TransGen Biotech, Beijing, China).
656 Then, we used PCR to amplify the sequences that covered *Gr10* or *Gr6* sgRNA
657 targeting sites. The reactions were 50 μL total and included: 2 μL template, 1 μL
658 forward primer, 1 μL reverse primer (mutation detection primer sequences are
659 provided in **Appendix—Table 5**), 25 μL Premix *Taq*™ (Takara, Dalian, China), and
660 19 μL ddH_2O. The procedure was as follows: 94°C for 3 min, 40 cycles [94°C for 30
661 s, 55°C (*Gr6*), or 60°C (*Gr10*) for 30 s], 72°C for 1 min, and finally 72°C for 5 min.
662 The PCR products were sent to Beijing Genomics Institute (BGI) or the
663 SinoGenoMax for Sanger sequencing, and the sequence results were analyzed by

664 SnapGene® software version 4.3.8 (from Insightful Science; available at
665 snapgene.com) and SeqMan software version 7.1 (DNASTAR, Madison, WI, USA).

666 **Screening homozygous mutants**

667 *Gr10* and *Gr6* homozygous mutant lines (*Gr10*$^{-/-}$ and *Gr6*$^{-/-}$, respectively) were
668 established. G0 mutants were backcrossed with the wild type to obtain G1 offspring.
669 For *Gr10*$^{-/-}$, *Gr10* heterozygotes of G1 moths, one DNA strands containing 4
670 bp-deleted modifications, were in-crossed to establish stable lines; for the expanding
671 mutant population, 4 bp-deleted homozygotes of G2 moths were in-crossed to
672 generate homozygotes G3 moths **(Figure 5A).** Similarly, for *Gr6*$^{-/-}$, *Gr6* heterozygotes
673 of G1 moths, one DNA strands containing 17 bp-deleted modifications, were
674 in-crossed to establish stable lines; for the expanding mutant population, 17
675 bp-deleted homozygotes of G2 moths were in-crossed to generate homozygotes G3
676 moths (**Figure 5D**). The related mutation rates are provided in **Appendix—Table 6.**

677 To confirm the deletion in *Gr10*$^{-/-}$ and *Gr6*$^{-/-}$ at mRNA level, the total RNA of the
678 female adult proboscis of WT, *Gr10*$^{-/-}$ and *Gr6*$^{-/-}$ were extracted, and the cDNA was
679 synthesized following the previous gene cloning protocol. The specific primers
680 (**Appendix—Table 5**) were designed based on the DNA exon sequences of *Gr10* and
681 *Gr6*, which covered the DNA-confirmed sgRNA targeting sites. During mRNA
682 sequence cloning, the PCR reaction conditions are largely the same as those used for
683 DNA sequence confirmation, with little modification (the annealing temperature
684 reduced to 50°C). The nucleic acid sequences of the identified *Gr10* and *Gr6* were
685 aligned manually by tools at the T-Coffee web server (Notredame et al., 2000).

686 **Off-target effects detection**

687 The potential off-target sites were predicted as previously described (Chen et al.,
688 2020). The online software Cas-OFFinder (http://www.rgenome.net/cas-offinder/) was
689 used to blast the *Gr10* and *Gr6* sgRNAs against the *H. armigera* genome (Pearce et
690 al., 2017), with mismatch number ≤ 3. According to the blast results, *Gr10* has no
691 potential off-target site, and one sequence (GenBank accession number:
692 LOC126054697) in the presence of *Gr6* sgRNA showed a potential off-target region.
693 The specific primers (**Appendix—Table 5**) were designed based on the DNA
694 sequence of LOC126054697 covering the potential off-target sites. The DNA
695 extraction and mutagenesis detection methods were described previously. Five
696 individual replicates were performed.

697 To further illustrate that the mutation strains did not exhibit off-target effects, the
698 electrophysiological responses of larvae and the biological parameters were compared
699 among WT, *Gr10*$^{-/-}$, and *Gr6*$^{-/-}$. The larval medial sensilla styloconica to xylose, the
700 body weight of the fifth instar larvae on the first day, the adult lifespan, and the
701 number of eggs laid were recorded. Five to sixteen individuals were recorded.

702 **Effects of *Gr10* or *Gr6* knockout on sugar reception in larvae and adults**

703 We recorded the electrophysiological responses of taste sensilla in the wild type (WT),
704 *Gr10*$^{-/-}$, and *Gr6*$^{-/-}$ by the tip-recording technique as described earlier. Concentration
705 of 1 mM, 10 mM, and 100 mM for sucrose and fucose were used as stimuli for lateral
706 sensilla styloconica in larvae. As the response thresholds of sugar GSNs in adults
707 were higher than those in larvae, concentrations of 10 mM and 100 mM for sucrose
708 and fucose and 100 mM for fructose were used for adult taste sensilla. 2 mM KCl was
709 used as the control for both larval and adult taste sensilla. 10 mM sinigrin and 10 mM

710 nicotine were used as a positive control (induced the response of a bitter cell in the
711 same sensilla) for larval and adult taste sensilla, respectively. All stimuli were tested
712 randomly, and 12–20 replicates were set for each experiment.

713 **Effects of *Gr10* or *Gr6* knockout on behavioral responses of larvae to sugars and**
714 **plant tissues**

715 The feeding preference of WT, *Gr10*$^{-/-}$, and *Gr6*$^{-/-}$ larvae to 10 mM and 100 mM
716 sucrose was investigated in two-choice tests as described earlier. 20 replicates were
717 set for each experiment. We also performed the no-choice tests to measure the feeding
718 amount of WT, *Gr10*$^{-/-}$, and *Gr6*$^{-/-}$ larvae in 1 h on leaves of cabbage (*Brassica*
719 *oleraceaas*, Shenglv-7), corn kernels of maize (*Zea mays,* Jingke-968), seeds of pea
720 (*Pisum sativum*, Changshouren), fruits of pepper (*Capsicum frutescens*, Changjian),
721 and fruits of tomato (*Solanum lycopersicum*, Ailsa Craig-LA2838A). Fifth instar
722 larvae on the first day after molting were starved for 2.5 h before testing. Each plant
723 tissue was weighed and put into a Petri dish (10 cm diameter × 2 cm depth; Corning,
724 Wujiang, China), and then one larva was introduced. The larva was removed after 1 h,
725 and the rest of the plant tissue was weighed. Petri dishes with plant tissues but no
726 caterpillar was used to measure water evaporation of the plant tissues. The feeding
727 amount of larvae is calculated based on the weight changes of the plant tissue before
728 and after being fed. 20 replicates were set for each experiment.

729 **Effects of *Gr10* or *Gr6* knockout on behavioral responses of adults to sugars**

730 The behavioral responses of the female adults of WT, *Gr10*$^{-/-}$, and *Gr6*$^{-/-}$ to sugars
731 were determined by using the PER test as described earlier. sucrose, fucose, and
732 fructose at the concentrations of 10 mM, 100 mM, and 1000 mM were tested

733 randomly to antennae and tarsi. H_2O was used as the control. All the experiments
734 were repeated three times, and 20 individual moths were used for each set of tests.

735 **Statistical analysis**

736 Statistical analyses were performed by SPSS 20 (IBM, Chicago, IL, USA) and
737 GraphPad Prism 8.2.1 (Dotmatics, San Diego, CA, USA). For the tip-recording data
738 and the PER data, the comparison between test compounds and the control was
739 performed using two-tailed independent-samples *t* test. For the two-choice feeding
740 test, paired-samples *t* test was used. The data of dose-response curves in tip-recording,
741 PER experiments and two-electrode voltage-clamp recording, and gene relative
742 expression levels and feeding amount in the no-choice test were analyzed by one-way
743 ANOVA with Tukey's HSD tests. The comparison among mutation strains and WT in
744 electrophysiological and PER experiments were analyzed by two-way ANOVA with
745 post hoc Tukey's multiple comparison. All analytical methods were tested at $P < 0.05$
746 for significant differences.

747

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Author Contributions

Conceptualization, C.Z.W. and S.S.Z.; Methodology, S.S.Z., P.C.W., L.Q.H., C.N., G.C.L., and C.Z.W.; Software, S.S.Z., P.C.W., C.N., G.C.L., and C.Z.W.; Validation, S.S.Z. and C.Z.W.; Formal Analysis, S.S.Z., C.N., G.C.L., and C.Z.W.; Investigation, S.S.Z., P.C.W., K.Y., L.L.C., L.Q.H., and C.Z.W.; Resources, S.S.Z., P.C.W., L.Q.H., and C.Z.W.; Data curation, S.S.Z., C.N., P.C.W., G.C.L., and C.Z.W.; Writing – original draft, C.Z.W. and S.S.Z.; Writing – review & editing, C.Z.W., S.S.Z.; Visualization, S.S.Z. and C.Z.W.; Supervision, C.Z.W.; Project administration, C.Z.W. and L.Q.H.; Funding acquisition, C.Z.W. and K.Y.

Competing interests

The authors declare no competing interests.

Data availability

All data and materials generated in this study are available in the manuscript, supplementary materials, Appendix, and source data. Sequence data that support the finding of this study have been deposited in GenBank with the accession numbers (see **Appendix—Table 1**).

References

Ai, D., Dong, C., Yang, B., Yu, C., & Wang, G. (2022). A fructose receptor gene influences development and feed intake in *Helicoverpa armigera*. *Insect Sci.*, ***29***(4), 993-1005. https://doi.org/10.1111/1744-7917.12984

Bernays, E. A., & Chapman, R. F. (1994). *Host-Plant Selection by Phytophagous Insects*. Chapman & Hall.

Blaney, W. M., & Simmonds, M. S. J. (1988). Food selection in adults and larvae of three species of Lepidoptera: a behavioral and electrophysiological study. *Entomol. Exp. Appl.*, ***49***(1-2), 111-121. https://doi.org/10.1111/j.1570-7458.1988.tb02482.x

Blaney, W. M., & Simmonds, M. S. J. (1990). A behavioural and electrophysiological study of the role of tarsal chemoreceptors in feeding by adults of Spodoptera, *Heliothis virescens* and *Helicoverpa armigera*. *J. Insect Physiol.*, ***36***(10), 743-756. https://doi.org/10.1016/0022-1910(90)90048-K

Briscoe, A. D., Macias-Munoz, A., Kozak, K. M., Walters, J. R., Yuan, F., Jamie, G. A., . . . Jiggins, C. D. (2013). Female behaviour drives expression and evolution of gustatory receptors in butterflies. *PLoS Genet.*, ***9***(7), e1003620. https://doi.org/10.1371/journal.pgen.1003620

Capella-Gutierrez, S., Silla-Martinez, J. M., & Gabaldon, T. (2009). trimAl: a tool for automated alignment trimming in large-scale phylogenetic analyses. *Bioinformatics*, ***25***(15), 1972-1973. https://doi.org/10.1093/bioinformatics/btp348

Chapman, R. F. (2003). Contact chemoreception in feeding by phytophagous insects.

Annu. Rev. Entomol., ***48***, 455-484. https://doi.org/10.1146/annurev.ento.48.091801.112629

Chen, W., Dong, Y. H., Saqib, H. S. A., Vasseur, L., Zhou, W. W., Zheng, L., Xu, X. J. (2020). Functions of duplicated glucosinolate sulfatases in the development and host adaptation of *Plutella xylostella*. *Insect Biochem. Mol. Biol.*, *119*, 103316. https://doi.org/10.1016/j.ibmb.2020.103316

Chen, Y., Wang, P. C., Zhang, S. S., Yang, J., Li, G. C., Huang, L. Q., & Wang, C. Z. (2022). Functional analysis of a bitter gustatory receptor highly expressed in the larval maxillary galea of *Helicoverpa armigera*. *PLoS Genet*, *18*(10), e1010455. https://doi.org/10.1371/journal.pgen.1010455

Dahanukar, A., Foster, K., van der Goes van Naters, W. M., & Carlson, J. R. (2001). A Gr receptor is required for response to the sugar trehalose in taste neurons of *Drosophila*. *Nat. Neurosci.*, ***4***(12), 1182-1186. https://doi.org/10.1038/nn765

Dahanukar, A., Lei, Y. T., Kwon, J. Y., & Carlson, J. R. (2007). Two Gr genes underlie sugar reception in *Drosophila*. *Neuron*, ***56***(3), 503-516. https://doi.org/10.1016/j.neuron.2007.10.024

Damak, S., Rong, M., Yasumatsu, K., Kokrashvili, Z., Varadarajan, V., Zou, S., Margolskee, R. F. (2003). Detection of sweet and umami taste in the absence of taste receptor T1r3. *Science*, ***301***(5634), 850-853. https://doi.org/10.1126/science.1087155

Değirmenci, L., Geiger, D., Rogé Ferreira, F. L., Keller, A., Krischke, B., Beye, M., Scheiner, R. (2020). CRISPR/Cas 9-mediated mutations as a new tool for studying

taste in Honeybees. *Chem. Senses*, ***45***(8), 655-666. https://doi.org/10.1093/chemse/bjaa063

Delventhal, R., & Carlson, J. R. (2016). Bitter taste receptors confer diverse functions to neurons. *Elife*, ***5***, e11181. https://doi.org/10.7554/eLife.11181

Dethier, V. G. (1973). Electrophysiological studies of gustation in Lepidopterous larvae. *J. Comp. Physiol.*, ***82***, 103-134. https://doi.org/10.1007/BF00696148

Engsontia, P., Sangket, U., Chotigeat, W., & Satasook, C. (2014). Molecular evolution of the odorant and gustatory receptor genes in Lepidopteran insects: implications for their adaptation and speciation. *J. Mol. Evol.*, ***79***(1-2), 21-39. https://doi.org/10.1007/s00239-014-9633-0

Faucheux, M. J. (2013). Sensillum types on the proboscis of the Lepidoptera: a review. *Ann. Soc. Entomol. Fr.*, ***49***(1), 73-90. https://doi.org/10.1080/00379271.2013.767514

Free, J. B. (1993). *Insect Pollination of Crops* (2 ed.). Academic Press.

Freeman, E. G., Wisotsky, Z., & Dahanukar, A. (2014). Detection of sweet tastants by a conserved group of insect gustatory receptors. *Proc. Natl. Acad. Sci. U. S. A.*, ***111***(4), 1598-1603. https://doi.org/10.1073/pnas.1311724111

Fujii, S., Yavuz, A., Slone, J., Jagge, C., Song, X., & Amrein, H. (2015). *Drosophila* sugar receptors in sweet taste perception, olfaction, and internal nutrient sensing. *Curr. Biol.*, ***25***(5), 621-627. https://doi.org/10.1016/j.cub.2014.12.058

Glendinning, J. I., Foley, C., Loncar, I., & Rai, M. (2009). Induced preference for host

plant chemicals in the tobacco hornworm: contribution of olfaction and taste. *J. Comp. Physiol. A*, ***195***(6), 591-601. https://doi.org/10.1007/s00359-009-0434-7

Guo, H., Cheng, T., Chen, Z., Jiang, L., Guo, Y., Liu, J., . . . Mita, K. (2017). Expression map of a complete set of gustatory receptor genes in chemosensory organs of *Bombyx mori*. *Insect Biochem. Mol. Biol.*, ***82***, 74-82. https://doi.org/10.1016/j.ibmb.2017.02.001

Harari, O. A., Dekel, A., Wintraube, D., Vainer, Y., Mozes-Koch, R., Yakir, E., Bohbot, J. D. (2023). A sucrose-specific receptor in *Bemisia tabaci* and its putative role in phloem feeding. *iScience*, *26*(5). https://doi.org/10.1016/j.isci.2023.106752

Hill, C. A., Fox, A. N., Pitts, R. J., Kent, L. B., Tan, P. L., Chrystal, M. A., Zwiebel, L. J. (2002). G protein-coupled receptors in *Anopheles gambiae*. *Science*, ***298***(5591), 176-178. https://doi.org/10.1126/science.1076196

Hodgson, E. S., Lettvin, J. Y., & Roeder, K. D. (1955). Physiology of a primary chemoreceptor unit. *Science*, ***122***(3166), 417-418. https://doi.org/10.1126/science.122.3166.417-a

Jiang, X. J., Ning, C., Guo, H., Jia, Y. Y., Huang, L. Q., Qu, M. J., & Wang, C. Z. (2015). A gustatory receptor tuned to D-fructose in antennal sensilla chaetica of *Helicoverpa armigera*. *Insect Biochem. Mol. Biol.*, ***60***, 39-46. https://doi.org/10.1016/j.ibmb.2015.03.002

Jiao, Y., Moon, S. J., & Montell, C. (2007). A *Drosophila* gustatory receptor required for the responses to sucrose, glucose, and maltose identified by mRNA tagging. *Proc.*

Natl. Acad. Sci. U. S. A., ***104***(35), 14110-14115. https://doi.org/10.1073/pnas.0702421104

Jiao, Y., Moon, S. J., Wang, X., Ren, Q., & Montell, C. (2008). Gr64f is required in combination with other gustatory receptors for sugar detection in *Drosophila*. *Curr. Biol.*, ***18***(22), 1797-1801. https://doi.org/10.1016/j.cub.2008.10.009

Jørgensen, K., Almaas, T. J., Marion-Poll, F., & Mustaparta, H. (2007). Electrophysiological characterization of responses from gustatory receptor neurons of sensilla chaetica in the moth *Heliothis virescens*. *Chem. Senses*, ***32***(9), 863-879. https://doi.org/10.1093/chemse/bjm057

Jung, J. W., Park, K. W., Ahn, Y. J., & Kwon, H. W. (2015). Functional characterization of sugar receptors in the western honeybee, *Apis mellifera*. *J. Asia Pac. Entomol.*, ***18***(1), 19-26. https://doi.org/10.1016/j.aspen.2014.10.011

Kent, L. B., Walden, K. K., & Robertson, H. M. (2008). The Gr family of candidate gustatory and olfactory receptors in the yellow-fever mosquito *Aedes aegypti*. *Chem. Senses*, ***33***(1), 79-93. https://doi.org/10.1093/chemse/bjm067

Kwon, J. Y., Dahanukar, A., Weiss, L. A., & Carlson, J. R. (2011). Molecular and cellular organization of the taste system in the *Drosophila* larva. *J. Neurosci.*, *31*(43), 15300-15309. https://doi.org/10.1523/jneurosci.3363-11.2011

Li, X., Staszewski, L., Xu, H., Durick, K., Zoller, M., & Adler, E. (2002). Human receptors for sweet and umami taste. *Proc. Natl. Acad. Sci. U. S. A.*, ***99***(7), 4692-4696. https://doi.org/10.1073/pnas.072090199

Liman, E. R., Zhang, Y. V., & Montell, C. (2014). Peripheral coding of taste. *Neuron*, ***81***(5), 984-1000. https://doi.org/10.1016/j.neuron.2014.02.022

Liu, X. L., Sun, S. J., Hou, W., Zhang, J., Yan, Q., & Dong, S. L. (2020). Functional characterization of two spliced variants of fructose gustatory receptor in the diamondback moth, *Plutella xylostella*. *Pestic. Biochem. Physiol.*, ***164***, 7-13. https://doi.org/10.1016/j.pestbp.2019.12.002

Liu, X. L., Yan, Q., Yang, Y. L., Hou, W., Miao, C. L., Peng, Y. C., & Dong, S. L. (2019). A gustatory receptor GR8 tunes specifically to D-fructose in the common cutworm *Spodoptera litura*. *Insects*, ***10***(9), 272. https://doi.org/10.3390/insects10090272

Livak, K. J., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ Method. *Methods*, ***25***(4), 402-408. https://doi.org/10.1006/meth.2001.1262

Maier, G. L., Komarov, N., Meyenhofer, F., Kwon, J. Y., & Sprecher, S. G. (2021). Taste sensing and sugar detection mechanisms in *Drosophila* larval primary taste center. *Elife*, *10*. https://doi.org/10.7554/eLife.67844

Mang, D., Mayu, K., Toyama, T., Yamagishi, T., & Sato, R. (2022). BmGr4 responds to sucrose and glucose and expresses in tachykinin-related peptide-secreting enteroendocrine cells. *Insect Biochem. Mol. Biol.*, ***150***, 103858. https://doi.org/10.1016/j.ibmb.2022.103858

Martin, T. L., & Shields, V. D. C. (2012). Detection of alkaloids and carbohydrates by

taste receptor cells of the galea of gypsy moth larvae, *Lymantria dispar* (L.). *Arthropod-Plant Interactions*, ***6***(4), 519-529. https://doi.org/10.1007/s11829-012-9209-0

Mishra, D., Miyamoto, T., Rezenom, Y. H., Broussard, A., Yavuz, A., Slone, J., Amrein, H. (2013). The molecular basis of sugar sensing in *Drosophila* larvae. *Curr. Biol.*, ***23***(15), 1466-1471. https://doi.org/10.1016/j.cub.2013.06.028

Miyamoto, T., Slone, J., Song, X. Y., & Amrein, H. (2012). A fructose receptor functions as a nutrient sensor in the *Drosophila* brain. *Cell*, ***151***(5), 1113-1125, Article 23178127. https://doi.org/10.1016/j.cell.2012.10.024

Morinaga, S., Nagata, K., Ihara, S., Yumita, T., Niimura, Y., Sato, K., & Touhara, K. (2022). Structural model for ligand binding and channel opening of an insect gustatory receptor. *J. Biol. Chem.*, ***298***(11), 102573. https://doi.org/10.1016/j.jbc.2022.102573

Nakagawa, T., & Touhara, K. (2013). Functional assays for insect olfactory receptors in *Xenopus* oocytes. In K. Touhara (Ed.), *Pheromone Signaling* (pp. 107-119). Humana Press. https://doi.org/10.1007/978-1-62703-619-1

Nelson, G., Hoon, M. A., Chandrashekar, J., Zhang, Y., Ryba, N. J., & Zuker, C. S. (2001). Mammalian sweet taste receptors. *Cell*, ***106***(3), 381-390. https://doi.org/10.1016/s0092-8674(01)00451-2

Nguyen, L. T., Schmidt, H. A., von Haeseler, A., & Minh, B. Q. (2015). IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood

920 phylogenies. *Mol. Biol. Evol.*, ***32***(1), 268-274.
921 https://doi.org/10.1093/molbev/msu300

922 Nicolson, S. W. (1998). The importance of osmosis in nectar secretion and its
923 consumption by insects. *Am. Zool.*, ***38***(3), 418-425.
924 https://doi.org/10.1093/icb/38.3.418

925 Notredame, C., Higgins, D. G., & Heringa, J. (2000). T-Coffee: A novel method for
926 fast and accurate multiple sequence alignment. *J. Mol. Biol.*, *302*(1), 205-217.
927 https://doi.org/10.1006/jmbi.2000.4042

928 Pearce, S. L., Clarke, D. F., East, P. D., Elfekih, S., Gordon, K. H. J., Jermiin, L. S.,
929 Wu, Y. D. (2017). Genomic innovations, transcriptional plasticity and gene loss
930 underlying the evolution and divergence of two highly polyphagous and invasive
931 *Helicoverpa* pest species. *BMC Biol.*, ***15***(1), 63.
932 https://doi.org/10.1186/s12915-017-0402-6

933 Popescu, A., Couton, L., Almaas, T. J., Rospars, J. P., Wright, G. A., Marion-Poll, F.,
934 & Anton, S. (2013). Function and central projections of gustatory receptor neurons
935 on the antenna of the noctuid moth *Spodoptera littoralis*. *J. Comp. Physiol. A*, ***199***(5),
936 403-416. https://doi.org/10.1007/s00359-013-0803-0

937 Robertson, H. M., & Wanner, K. W. (2006). The chemoreceptor superfamily in the
938 honey bee, *Apis mellifera*: expansion of the odorant, but not gustatory, receptor
939 family. *Genome Res.*, ***16***(11), 1395-1403. https://doi.org/10.1101/gr.5057506

940 Robertson, H. M., Warr, C. G., & Carlson, J. R. (2003). Molecular evolution of the

insect chemoreceptor gene superfamily in *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. U. S. A.*, ***100*** 14537-14542. https://doi.org/10.1073/pnas.2335847100

Rozewicki, J., Li, S., Amada, K. M., Standley, D. M., & Katoh, K. (2019). MAFFT-DASH: integrated protein sequence and structural alignment. *Nucleic Acids Res.*, ***47***(W1), W5-W10. https://doi.org/10.1093/nar/gkz342

Sato, K., Tanaka, K., & Touhara, K. (2011). Sugar-regulated cation channel formed by an insect gustatory receptor. *Proc. Natl. Acad. Sci. U. S. A.*, ***108***(28), 11680-11685. https://doi.org/10.1073/pnas.1019622108

Schauer, N., Zamir, D., & Fernie, A. R. (2005). Metabolic profiling of leaves and fruit of wild species tomato: a survey of the *Solanum lycopersicum* complex. *J. Exp. Bot.*, ***56***(410), 297-307. https://doi.org/10.1093/jxb/eri057

Schnuch, M., & Seebauer, H. (1998). Sugar cell responses to lactose and sucrose in labellar and tarsal taste hairs of *Musca domestica*. *J. Comp. Physiol. A*, ***182***(6), 767-775. https://doi.org/10.1007/s003590050221

Schoonhoven, L. M., & van Loon, J. J. A. (2002). An inventory of taste in caterpillars: each species its own key. *Acta ZooI. Acad. Sci. Hungaricae*, ***48***, 215-263.

Schoonhoven, L. M., van Loon, J. J. A., & Dicke, M. (2005). *Insect-Plant Biology* (2 ed.). Oxford University Press.

Sharath Chandra, G., Asokan, R., Manamohan, M., Krishna Kumar, N. K., & Sita, T. (2014). Evaluation of reference genes for quantitative real-time PCR normalization in cotton bollworm *Helicoverpa armigera*. *Mol. Biol. (Mosk)*, *48*, 813-822.

Slone, J., Daniels, J., & Amrein, H. (2007). Sugar receptors in *Drosophila*. *Curr. Biol.*, ***17***(20), 1809-1816. https://doi.org/10.1016/j.cub.2007.09.027

Smith, J. J. B., Mitchell, B. K., Rolseth, B. M., Whitehead, A. T., & Albert, P. J. (1990). SAPID tools - microcomputer programs for analysis of multiunit nerve recordings. *Chem. Senses*, ***15***(3), 253-270. https://doi.org/10.1093/chemse/15.3.253

Takada, T., Sasaki, T., Sato, R., Kikuta, S., & Inoue, M. N. (2018). Differential expression of a fructose receptor gene in honey bee workers according to age and behavioral role. *Arch. Insect Biochem. Physiol.*, ***97***(2), 1-9. https://doi.org/10.1002/arch.21437

Tang, D. L., Wang, C. Z., Luo, L., & Qin, J. D. (2000). Comparative study on the responses of maxillary sensilla styloconica of cotton bollworm *Helicoverpa armigera* and Oriental tobacco budworm *H. assulta* larvae to phytochemicals. *Sci. China Life Sci.* , ***43***(6), 606-612. https://doi.org/10.1007/Bf02882281

van Loon, J. J. A. (1990). Chemoreception of phenolic acids and flavonoids in larvae of two species of *Pieris*. *J. Comp. Physiol. A*, ***166***(6), 889-899. https://doi.org/10.1007/bf00187336

Vansell, G. H. (1944). Cotton nectar in relation to bee activity and honey production. *J. Econ. Entomol.*, ***37***, 528-530. https://doi.org/10.1093/jee/37.4.528

Waller, G. D., Wilson, F. D., & Martin, J. H. (1981). Influence of phenotype, season, and time-of-day on nectar production in cotton. *Crop Sci.*, ***21***(4), 507-511. https://doi.org/10.2135/cropsci1981.0011183X002100040007x

Xu, W., Liu, N., Liao, Y., & Anderson, A. (2017). Molecular characterization of sugar taste receptors in the cotton bollworm *Helicoverpa armigera*. *Genome*, ***60***(12), 1037-1044. https://doi.org/10.1139/gen-2017-0086

Xu, W., Zhang, H. J., & Anderson, A. (2012). A sugar gustatory receptor identified from the foregut of cotton bollworm *Helicoverpa armigera*. *J. Chem. Ecol.*, ***38***(12), 1513-1520. https://doi.org/10.1007/s10886-012-0221-8

Yarmolinsky, D. A., Zuker, C. S., & Ryba, N. J. P. (2009). Common sense about taste: from mammals to insects. *Cell*, ***139***(2), 234-244. https://doi.org/10.1016/j.cell.2009.10.001

Zhan, S., Merlin, C., Boore, J. L., & Reppert, S. M. (2011). The monarch butterfly genome yields insights into long-distance migration. *Cell*, ***147***(5), 1171-1185. https://doi.org/10.1016/j.cell.2011.09.052

Zhang, H. J., Faucher, C. P., Anderson, A., Berna, A. Z., Trowell, S., Chen, Q. M., Chyb, S. (2013). Comparisons of contact chemoreception and food acceptance by larvae of polyphagous *Helicoverpa armigera* and oligophagous *Bombyx mori*. *J. Chem. Ecol.*, ***39***(8), 1070-1080. https://doi.org/10.1007/s10886-013-0303-2

Zhang, Y. F., van Loon, J. J. A., & Wang, C. Z. (2010). Tarsal taste neuron activity and proboscis extension reflex in response to sugars and amino acids in *Helicoverpa armigera* (Hübner). *J. Exp. Biol.*, ***213***(16), 2889-2895. https://doi.org/10.1242/jeb.042705

Zhao, G. Q., Zhang, Y., Hoon, M. A., Chandrashekar, J., Erlenbach, I., Ryba, N. J., &

Zuker, C. S. (2003). The receptors for mammalian sweet and umami taste. *Cell*, ***115***(3), 255-266. https://doi.org/10.1016/s0092-8674(03)00844-4

1007 **Figure legends**

1008 **Figure 1. Electrophysiological responses of larval and adult taste sensilla in**
1009 ***Helicoverpa armigera* to sugars. (A)** The representative spike traces of the responses
1010 of lateral sensilla styloconica in larval maxillary galea to seven sugars at 10 mM (left),
1011 and quantifications of the firing rates (right) (n = 13). **(B)** Dose–responses of lateral
1012 sensilla styloconica in larval maxillary galea to sucrose and fucose (sucrose: n = 8;
1013 fucose: n = 6). **(C)** The representative spike traces of the responses of sensilla
1014 chaetica in adult antennae to seven sugars at 10 mM (left), and quantifications of the
1015 firing rates (right) (n = 21). **(D)** Dose–responses of sensilla chaetica in adult antennae
1016 to sucrose, fucose, and fructose (n = 21). **(E)** The representative spike traces of the
1017 responses of sensilla trichodea in adult tarsi to seven sugars at 10 mM (left), and
1018 quantifications of the firing rates (right) (n = 15). **(F)** Dose–responses of sensilla
1019 trichodea in adult tarsi to sucrose, fucose, and fructose (n = 21). **(G)** The
1020 representative spike traces of the responses of sensilla styloconica in adult proboscis
1021 to seven sugars at 10 mM (left), and quantifications of the firing rates (right) (n = 21).
1022 **(H)** Dose–responses to sucrose (n = 21), fucose (n = 21), and fructose (fructose 0 mM,
1023 n = 18; fructose 0.01 mM-100 mM, n = 21) of sensilla styloconica in adult proboscis.
1024 **(A to H)** Data are mean ± SEM; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. **(A, C, E, G)**
1025 Data were analyzed by independent-samples t test (compared with control). **(B, D, F,**
1026 **H)** Data were analyzed by one-way ANOVA with Tukey's HSD test.

1027 **Figure 2. Behavioral responses of *Helicoverpa armigera* larvae and adults to**
1028 **sugars. (A)** Feeding responses and the preference index (PI) value of 5th instar larvae
1029 to seven sugars painted on the cabbage leaf discs at 10 mM in two-choice tests. ** P
1030 < 0.01; ns indicates no significance, $P \geq 0.05$ (paired t test, n = 20). **(B)** Feeding

1031 responses and the PI value of 5th instar larvae to different concentrations of sucrose
1032 painted on the cabbage leaf discs in two-choice tests. ** $P < 0.01$, *** $P < 0.001$; ns
1033 indicates no significance, $P \geq 0.05$ (paired *t* test, $n = 20$). **(C)** Proboscis extension
1034 reflex (PER) in adult females upon antennal stimulation by seven sugars at 100 mM.
1035 ** $P < 0.01$; *** $P < 0.001$ (independent-samples *t* test compared with control, $n = 3$).
1036 **(D)** PER in adult females upon tarsal stimulation by seven sugars at 100 mM. * $P <$
1037 0.05; ** $P < 0.01$ (independent-samples *t* test, $n = 3$). **(E)** PER in adult females upon
1038 antennal stimulation by different concentrations of sucrose, fucose, and fructose. * P
1039 < 0.05 (one-way ANOVA with Tukey's HSD test, $n = 3$). **(F)** PER in adult females
1040 upon tarsal stimulation by different concentrations of sucrose, fucose, and fructose (n
1041 $= 3$). * $P < 0.05$ (one-way ANOVA with Tukey's HSD test, $n = 3$). **(A to F)** Data are
1042 mean ± SEM.

1043 **Figure 3. The phylogenetic relationship and the expression level of sugar GRs in**
1044 **larval maxilla and female adult antennae, tarsi and proboscis of *Helicoverpa***
1045 ***armigera*. (A)** The phylogenetic tree of insect sugar GRs. Diptera (orange): Aaeg,
1046 *Aedes aegypti*; Agam, A*nopheles gambiae*; Dmel, *Drosophila melanogaster*.
1047 Hymenoptera (blue): Am, *Apis mellifera*. Lepidoptera (black): Bmor, *Bombyx mori*;
1048 Dple, *Danaus plexippus*; Hmel, *Heliconius melpomene*; Pxyl, *Plutella xylostella*.
1049 Harm (red), *Helicoverpa armigera*. Numbers above branches indicate ultrafast
1050 bootstrap approximation (UFBoot). **(B)** Relative expression levels of sugar GRs in the
1051 maxillary galea of 5th instar larvae of *H. armigera* determined by qRT-PCR. **(C)**
1052 Relative expression levels of sugar GRs in the female adult antennae. **(D)** Relative
1053 expression levels of sugar GRs in female adult tarsi. **(E)** Relative expression levels of
1054 sugar GRs in female adult proboscis. **(B to E)** Data are mean ± SEM. One-way

1055 ANOVA was used, and different letters labeled indicate significant difference
1056 (Tukey's HSD test, $P < 0.05$, $n = 3$).

1057 **Figure 4. The inward current responses of the *Xenopus* oocytes expressing sugar**
1058 **GRs of *Helicoverpa armigera* to sugar compounds**. **(A)** The representative traces of
1059 the oocytes expressing Gr10, Gr6, and Gr10 + Gr6 to 11 sugar compounds at 100 mM.
1060 **(B)** The responses of the oocytes expressing Gr10, Gr6, and Gr10 + Gr6 to sugars at
1061 100 mM (Gr10, $n = 14$. Gr6, $n = 20$. Gr10 + Gr6, $n = 7$). **(C)** The representative trace
1062 of the oocytes expressing Gr10 to sucrose (the upper), the representative trace of the
1063 oocytes expressing Gr6 to sucrose, fucose, and fructose (the middle), the
1064 representative trace of the oocytes expressing Gr10 + Gr6 to sucrose and fucose (the
1065 lower). **(D)** The dose-responses of the oocytes expressing Gr10 to sucrose (the upper,
1066 $n = 13$); the dose-responses of the oocytes expressing Gr6 to sucrose, fucose, and
1067 fructose (the middle, sucrose: $n = 6$; fucose: $n = 6$; fructose: $n = 3$); the
1068 dose-responses of the oocytes expressing Gr10 + Gr6 to sucrose and fucose (the lower,
1069 sucrose: $n = 4$; fucose: $n = 3$). **(B and D)** Data are mean ± SEM, and were analyzed
1070 by one-way ANOVA with Tukey's HSD test ($P < 0.05$). Different letters labeled
1071 indicate significant differences, * $P < 0.05$. **(A to D)** Ara, arabinose; Fru, fructose;
1072 Fuc, fucose; Gal, galactose; Glu, glucose; Lac, lactose; Mal, maltose; Man, mannose;
1073 Suc, sucrose; Tre, trehalose; Xyl, xylose; Rin, ringer solution.

1074 **Figure 5. Establishment of *Gr10* and *Gr6* homozygous mutants (*Gr10*$^{-/-}$ and *Gr6*$^{-/-}$)**
1075 **of *Helicoverpa armigera* via CRISPR/Cas9**. **(A)** The cross process of obtaining
1076 *Gr10*$^{-/-}$. **(B)** The genomic structure of *Gr10*, the single-guide RNA (sgRNA) targeting
1077 sequence (in green), and representative chromatograms of direct sequencing of the
1078 PCR products obtained from wild types (WT) and *Gr10*$^{-/-}$, in which 4-bp of the *Gr10*

1079 sequence were deleted. **(C)** The predicted secondary structures of the Gr10 protein in
1080 WT and the truncated Gr10 protein in *Gr10*$^{-/-}$. **(D)** The cross process of obtaining
1081 *Gr6*$^{-/-}$. **(E)** The genomic structure of *Gr6*, the single-guide RNA (sgRNA) targeting
1082 sequence (in green), and representative chromatograms of direct sequencing of the
1083 PCR products obtained from WT and *Gr6*$^{-/-}$, in which 17-bp of the *Gr6* sequence were
1084 deleted. **(F)** The predicted secondary structures of the Gr6 protein in WT and the
1085 truncated Gr6 protein in *Gr6*$^{-/-}$. **(B and D)** Boxes represent exons, black lines
1086 represent introns, the green arrowhead indicates the direction of sgRNA; the
1087 protospacer adjacent motif (PAM) is in blue. **(C and F)** The secondary structure of
1088 *Gr10* and *Gr6* in WT, *Gr10*$^{-/-}$ and *Gr6*$^{-/-}$ was predicted by
1089 https://dtu.biolib.com/DeepTMHMM, and the image was constructed by TOPO2
1090 software (http://www.sacs.ucsf.edu/TOPO2).

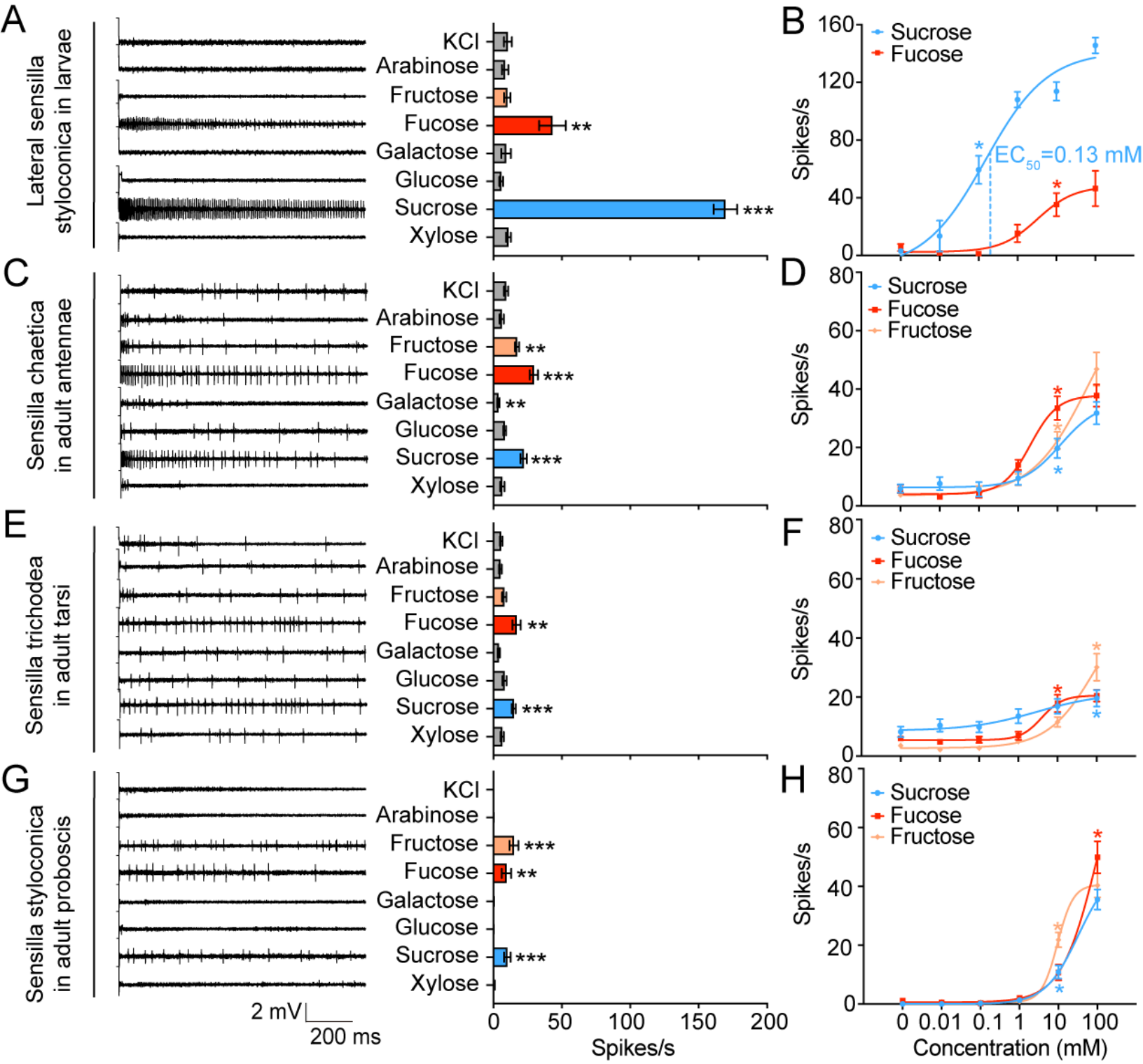
1091 **Figure 6. Electrophysiological responses of larval and adult taste sensilla in WT,**
1092 ***Gr10*$^{-/-}$ and *Gr6*$^{-/-}$ of *Helicoverpa armigera* to sucrose and other compounds. (A)**
1093 The representative spike traces of lateral sensilla styloconica in larval maxillary galea.
1094 **(B)** Quantifications of the firing rates of lateral sensilla styloconica in larval maxillary
1095 galea (mean ± SEM; WT, n = 12; *Gr6*$^{-/-}$, n = 13; *Gr10*$^{-/-}$, n = 13). **(C)** The
1096 representative spike traces of sensilla chaetica in female antennae. **(D)** Quantifications
1097 of the firing rates of sensilla chaetica in female antennae (mean ± SEM; WT, n = 20.
1098 *Gr6*$^{-/-}$: nicotine, n = 17; other compounds, n = 18. *Gr10*$^{-/-}$, n = 20). **(E)** The
1099 representative spike traces of sensilla trichodea in female tarsi. **(F)** Quantifications of
1100 the firing rates of sensilla trichodea in female tarsi (mean ± SEM; WT, n = 18; *Gr6*$^{-/-}$:
1101 nicotine, n = 14. other compounds, n = 18. *Gr10*$^{-/-}$, n = 18). **(G)** The representative
1102 spike traces of sensilla styloconica in female proboscis. **(H)** Quantifications of the

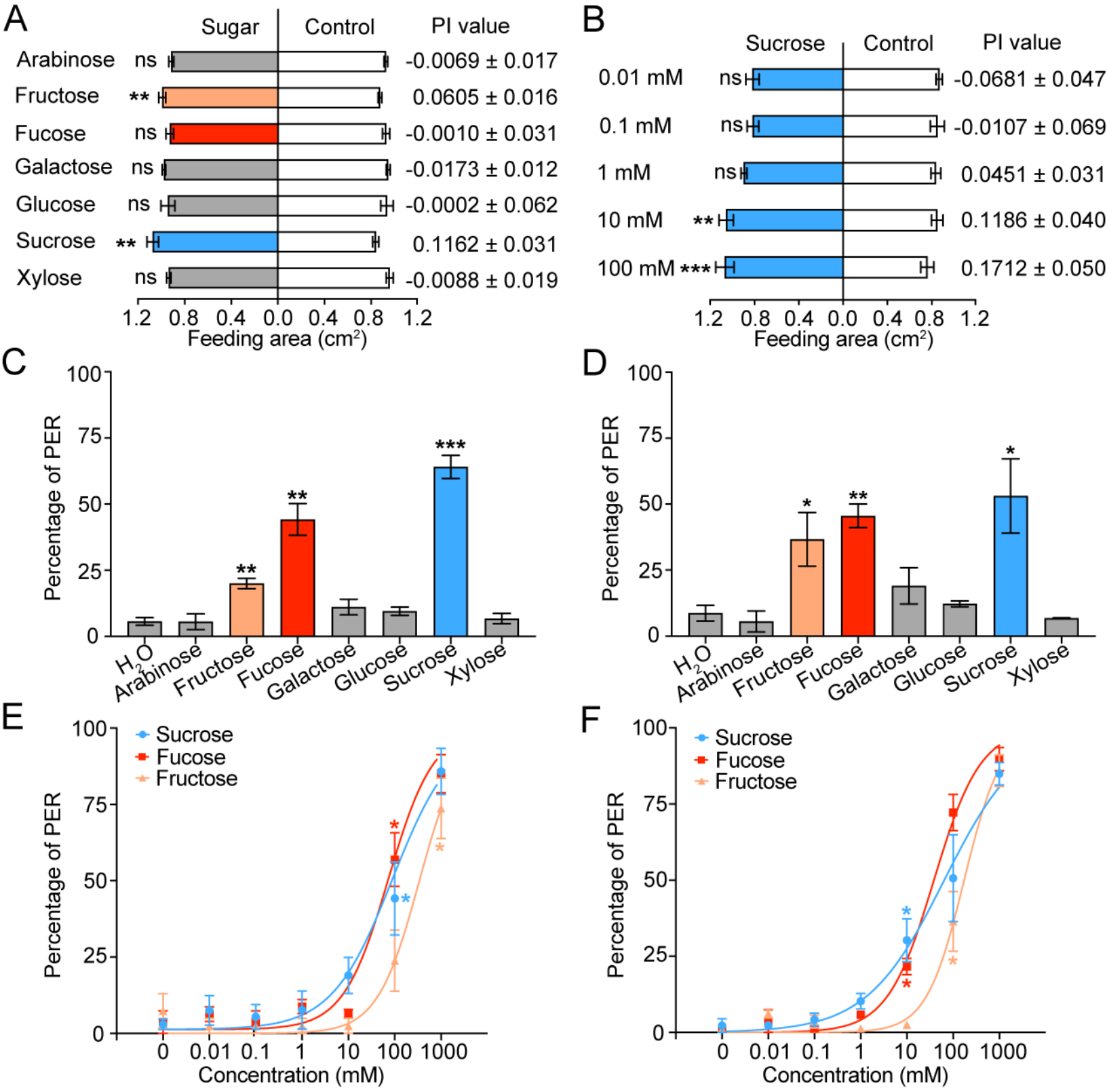
1103 firing rates of sensilla styloconica in female proboscis (mean ± SEM; WT: sucrose
1104 100 mM, n = 17; other compounds, n = 18. *Gr6*$^{-/-}$: sucrose 100 mM, n = 17; other
1105 compounds, n = 18. *Gr10*$^{-/-}$, n = 18). **(B, D, F, and H)** Two-way ANOVA with post
1106 hoc Tukey's multiple comparison was used separately for sucrose and fucose, and
1107 one-way ANOVA with Tukey's HSD test was used for KCl, sinigrin, fructose, and
1108 nicotine. Different letters labeled indicate significant differences (P < 0.05). Suc:
1109 sucrose; Fuc: fucose; Sin: sinigrin; Fru: fructose; Nic: nicotine.

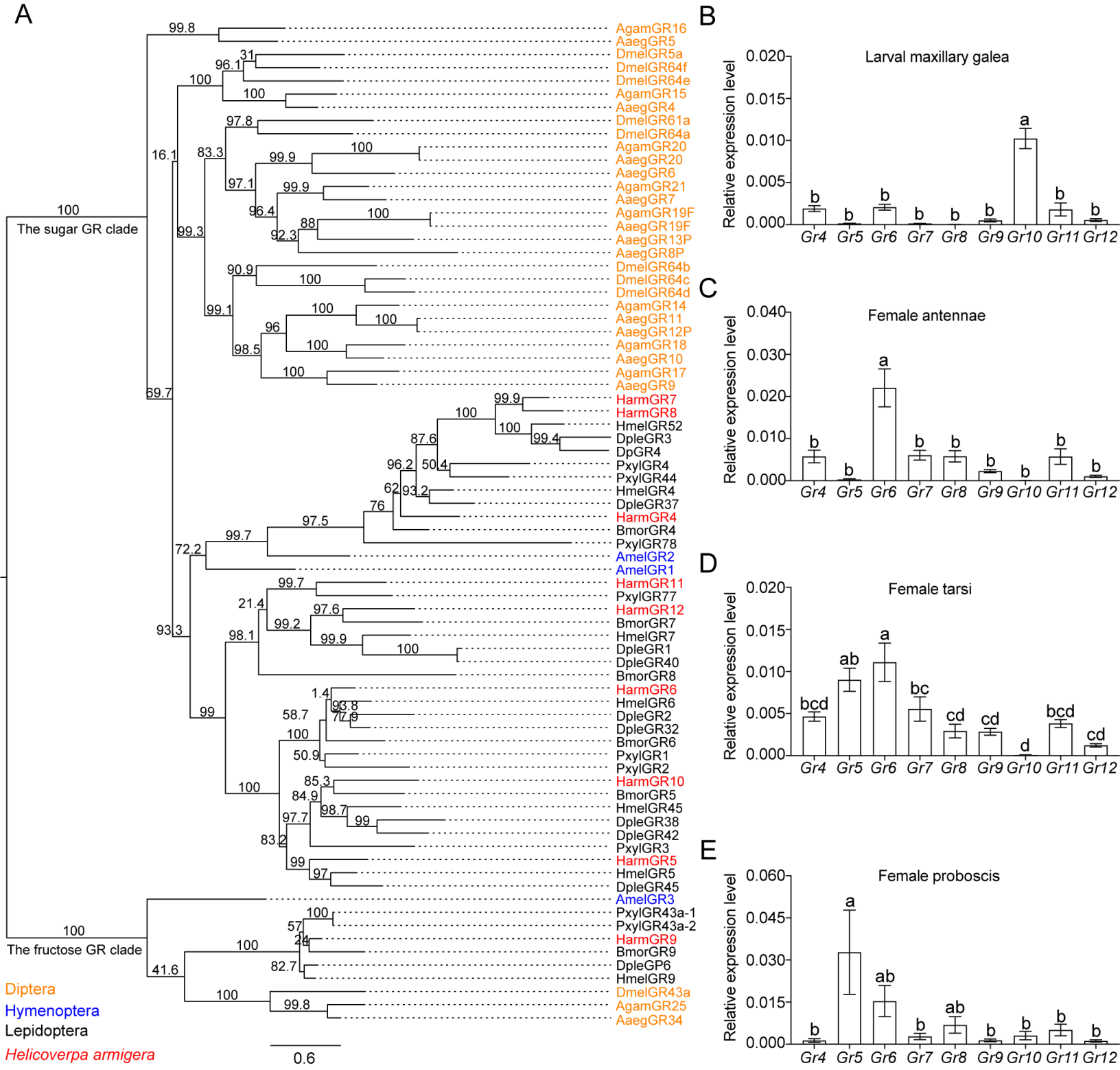
1110 **Figure 7. Behavioral responses of WT, *Gr10*$^{-/-}$ and *Gr6*$^{-/-}$ larvae and adults of**
1111 ***Helicoverpa armigera* to sugars and plant tissues. (A)** Feeding area of 5th instar
1112 larvae in two-choice tests and the PI value to 10 mM sucrose (n = 20). ** P < 0.01; ns
1113 indicates no significance, $P \geq 0.05$ (paired t test). Suc, sucrose. **(B)** Feeding area of
1114 5th instar larvae in two-choice tests and the PI value to 100 mM sucrose (n = 20). * P
1115 < 0.05; ** P < 0.01; *** P < 0.001 (paired t test). Suc, sucrose. **(C)** Feeding amount
1116 of 5th instar larvae on cabbage leaves, corn kernels, pea seeds, pepper fruits, and
1117 tomato fruits in no-choice tests (n = 20). Data were analyzed by one-way ANOVA
1118 with Tukey's HSD test, and different letters labeled on the data of WT, *Gr10*$^{-/-}$ and
1119 *Gr6*$^{-/-}$ for each plant tissue indicate significant differences (P < 0.05). **(D)** Proboscis
1120 extension reflex (PER) in adult females upon antennal stimulation by sucrose
1121 concentrations (n = 3). **(E)** PER in adult females upon antennal stimulation by fucose
1122 concentrations (n = 3). **(F)** PER in adult females upon antennal stimulation by
1123 fructose concentrations (n = 3). **(G)** PER in adult females upon tarsal stimulation by
1124 sucrose concentrations (n = 3). **(H)** PER in adult females upon tarsal stimulation by
1125 fucose concentrations (n = 3). **(I)** PER in adult females upon tarsal stimulation by
1126 fructose concentrations (n = 3). **(A to I)** Data are mean ± SEM. **(D to I)** Data were

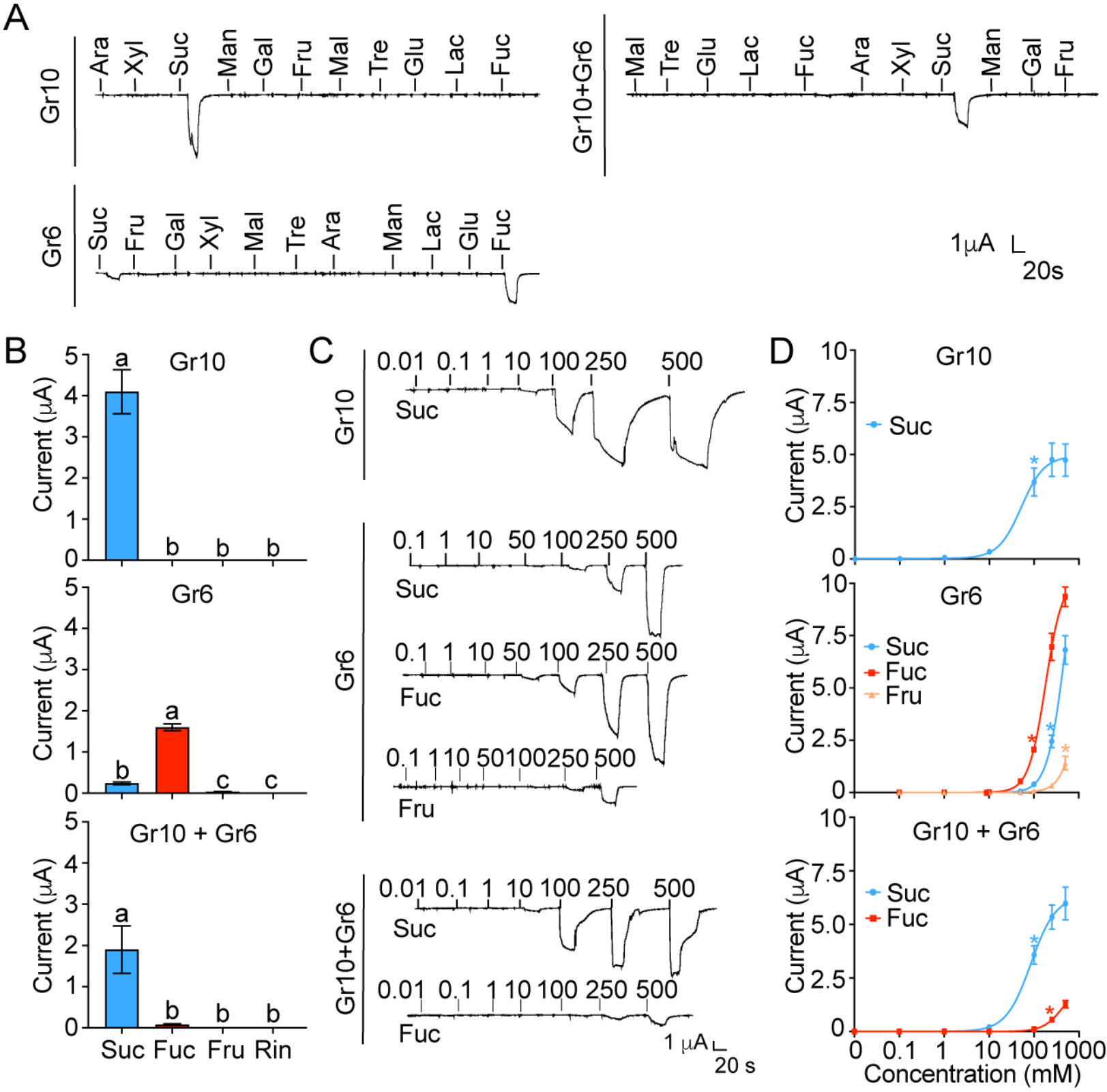
1127 analyzed by two-way ANOVA with post hoc Tukey's multiple comparison. * $P <$

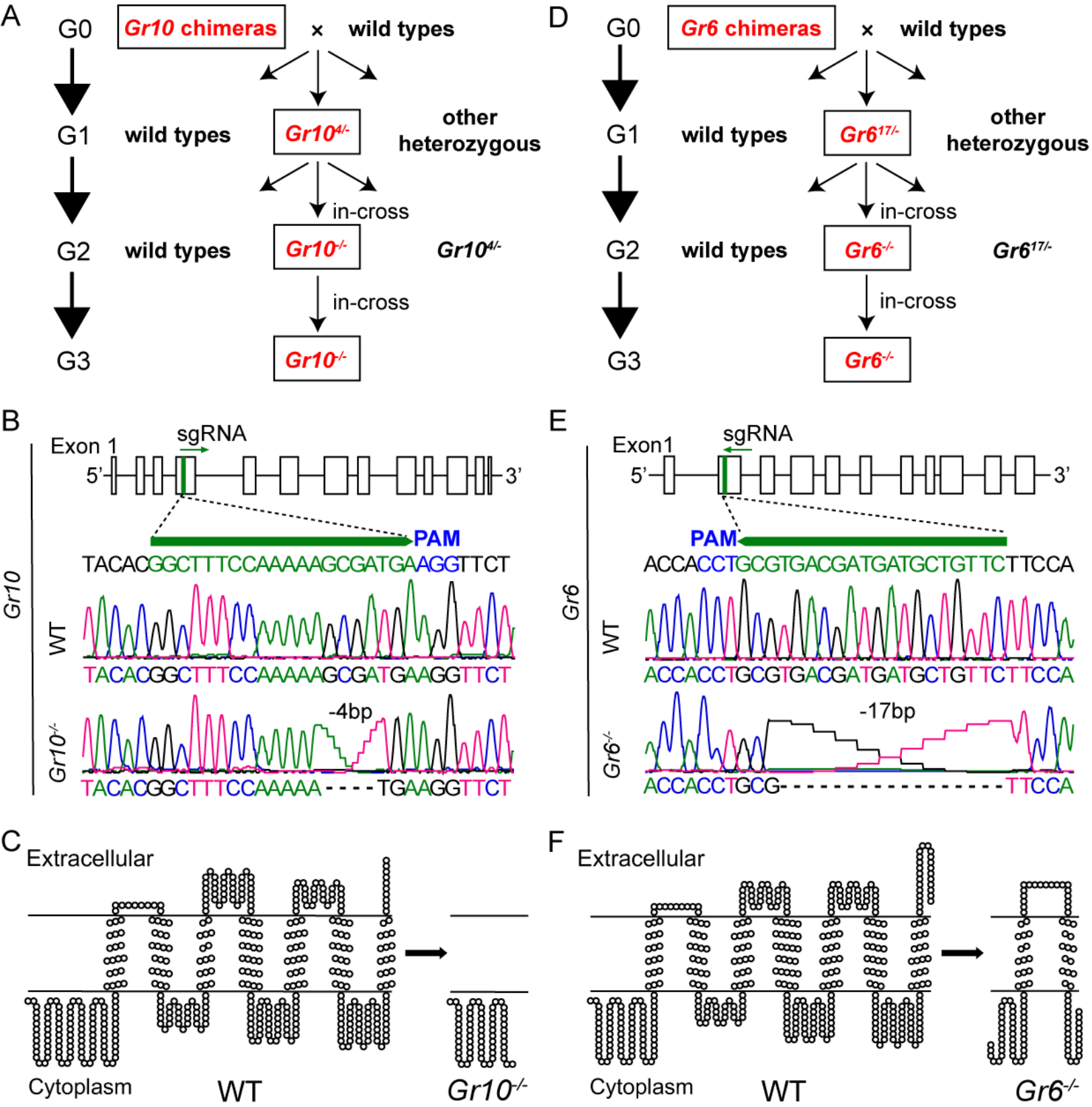
1128 0.05.

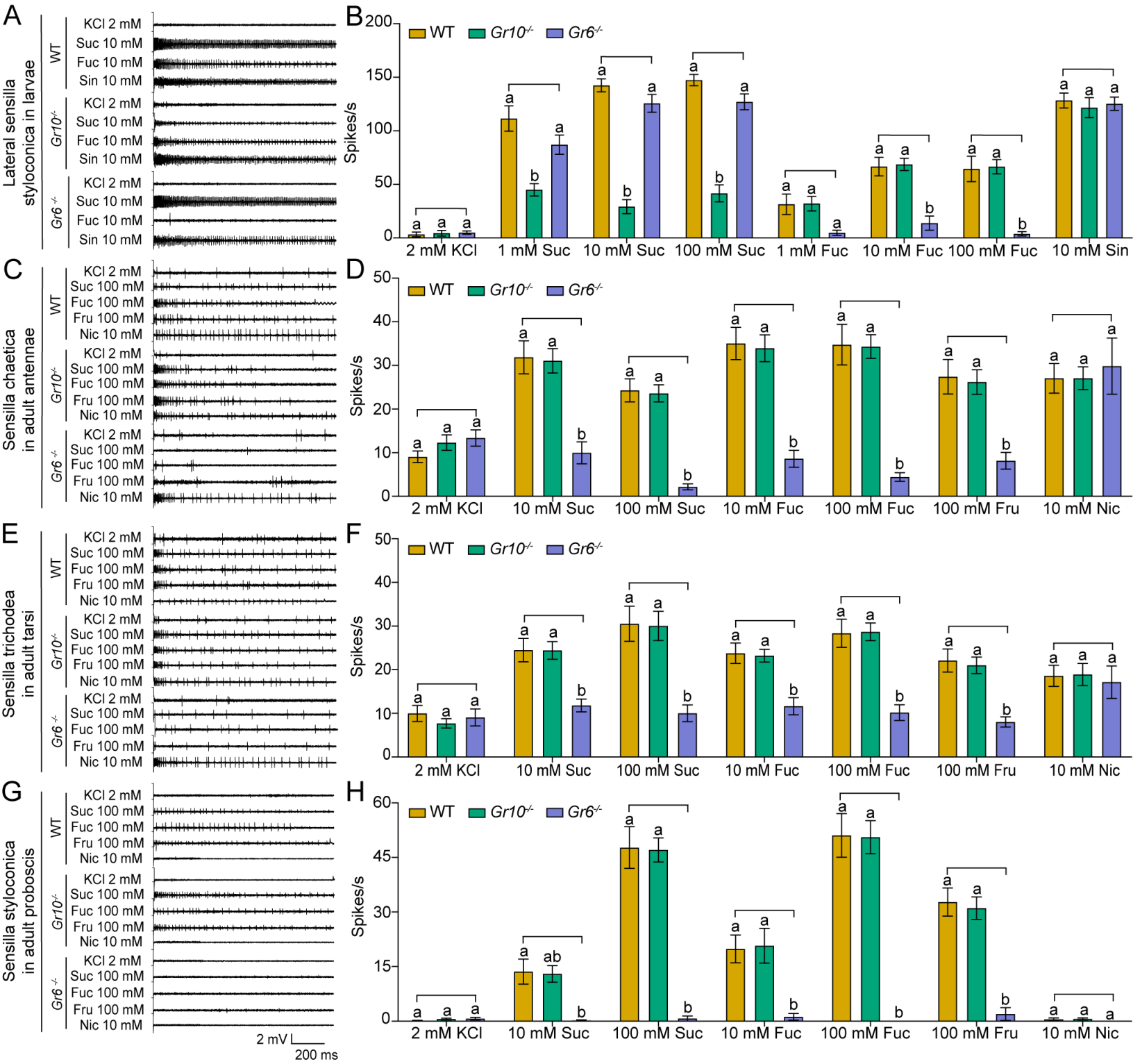

A
Lateral sensilla styloconica in larvae
KCl
Arabinose
Fructose
Fucose
Galactose
Glucose
Sucrose
Xylose
**
***
B
Sucrose
Fucose
EC50=0.13 mM
Spikes/s
C
Sensilla chaetica in adult antennae
Fructose
Fucose
Sucrose
D
Sucrose
Fucose
Fructose
E
Sensilla trichodea in adult tarsi
F
G
Sensilla styloconica in adult proboscis
H
2 mV
200 ms
0
50
100
150
200
Spikes/s
0 0.01 0.1 1 10 100
Concentration (mM)

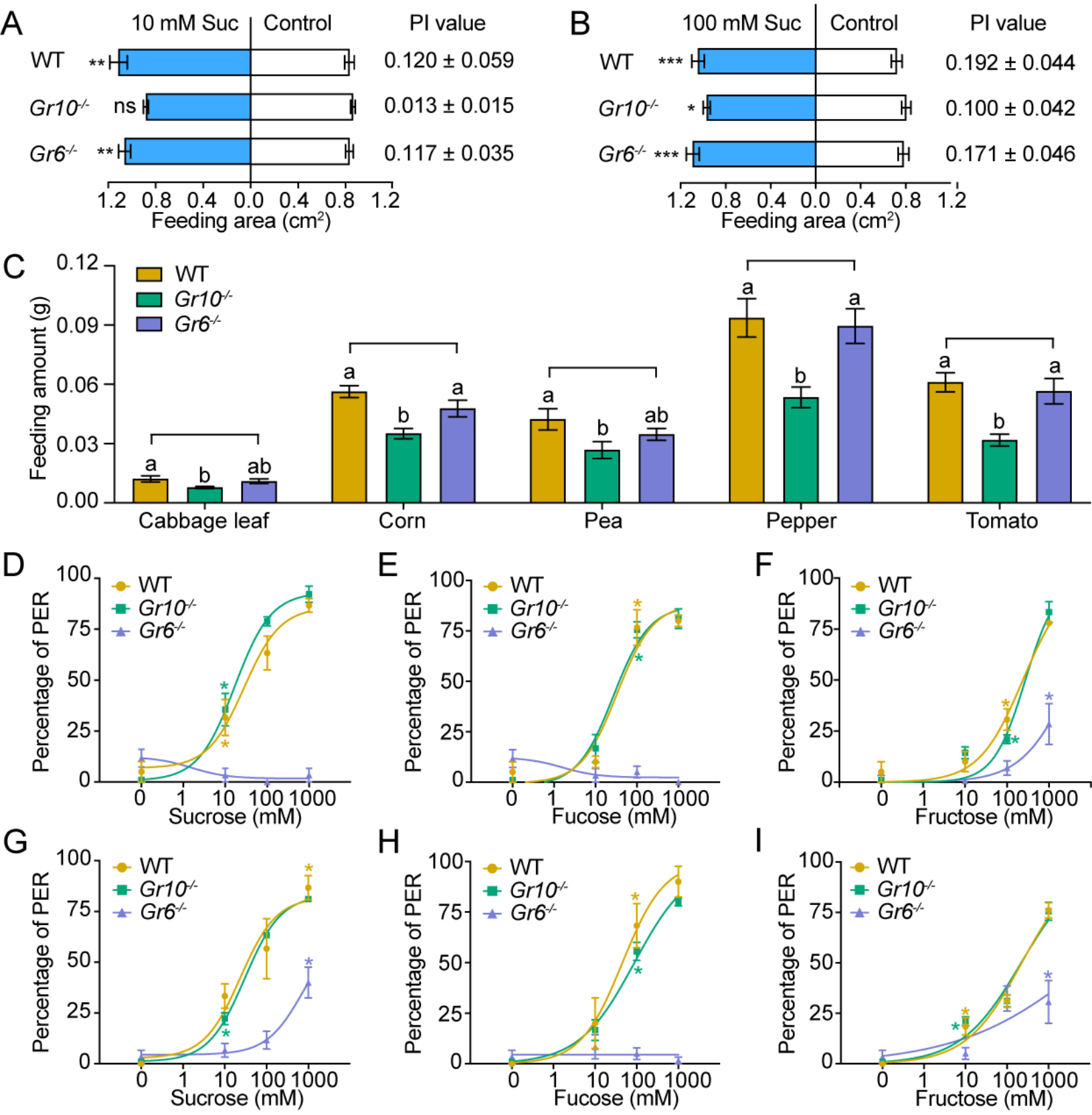

A
Sugar
Control
PI value
Arabinose ns -0.0069 ± 0.017
Fructose ** 0.0605 ± 0.016
Fucose ns -0.0010 ± 0.031
Galactose ns -0.0173 ± 0.012
Glucose ns -0.0002 ± 0.062
Sucrose ** 0.1162 ± 0.031
Xylose ns -0.0088 ± 0.019
1.2 0.8 0.4 0.0 0.4 0.8 1.2
Feeding area (cm²)
B
Sucrose
Control
PI value
0.01 mM ns -0.0681 ± 0.047
0.1 mM ns -0.0107 ± 0.069
1 mM ns 0.0451 ± 0.031
10 mM ** 0.1186 ± 0.040
100 mM *** 0.1712 ± 0.050
1.2 0.8 0.4 0.0 0.4 0.8 1.2
Feeding area (cm²)
C
Percentage of PER
100
75
50
25
0
H2O
Arabinose
Fructose
Fucose
Galactose
Glucose
Sucrose
Xylose
D
Percentage of PER
E
Sucrose
Fucose
Fructose
Percentage of PER
0 0.01 0.1 1 10 100 1000
Concentration (mM)
F
Sucrose
Fucose
Fructose
Percentage of PER
0 0.01 0.1 1 10 100 1000
Concentration (mM)


A
The sugar GR clade
The fructose GR clade
AgamGR16
AaegGR5
DmelGR5a
DmelGR64f
DmelGR64e
AgamGR15
AaegGR4
DmelGR61a
DmelGR64a
AgamGR20
AaegGR20
AaegGR6
AgamGR21
AaegGR7
AgamGR19F
AaegGR19F
AaegGR13P
AaegGR8P
DmelGR64b
DmelGR64c
DmelGR64d
AgamGR14
AaegGR11
AaegGR12P
AgamGR18
AaegGR10
AgamGR17
AaegGR9
HarmGR7
HarmGR8
HmelGR52
DpleGR3
DpGR4
PxylGR4
PxylGR44
HmelGR4
DpleGR37
HarmGR4
BmorGR4
PxylGR78
AmelGR2
AmelGR1
HarmGR11
PxylGR77
HarmGR12
BmorGR7
HmelGR7
DpleGR1
DpleGR40
BmorGR8
HarmGR6
HmelGR6
DpleGR2
DpleGR32
BmorGR6
PxylGR1
PxylGR2
HarmGR10
BmorGR5
HmelGR45
DpleGR38
DpleGR42
PxylGR3
HarmGR5
HmelGR5
DpleGR45
AmelGR3
PxylGR43a-1
PxylGR43a-2
HarmGR9
BmorGR9
DpleGP6
HmelGR9
DmelGR43a
AgamGR25
AaegGR34
Diptera
Hymenoptera
Lepidoptera
Helicoverpa armigera
0.6
B
Larval maxillary galea
C
Female antennae
D
Female tarsi
E
Female proboscis
Relative expression level
Gr4 Gr5 Gr6 Gr7 Gr8 Gr9 Gr10 Gr11 Gr12


A
Gr10
Ara
Xyl
Suc
Man
Gal
Fru
Mal
Tre
Glu
Lac
Fuc
Gr10+Gr6
Mal
Tre
Glu
Lac
Fuc
Ara
Xyl
Suc
Man
Gal
Fru
Gr6
Suc
Fru
Gal
Xyl
Mal
Tre
Ara
Man
Lac
Glu
Fuc
1μA
20s
B
Gr10
Gr6
Gr10 + Gr6
Current (μA)
Suc Fuc Fru Rin
C
Gr10
Gr6
Gr10+Gr6
0.01 0.1 1 10 100 250 500
Suc
Fuc
Fru
1 μA
20 s
D
Gr10
Gr6
Gr10 + Gr6
Suc
Fuc
Fru
Current (μA)
Concentration (mM)


A
G0
Gr10 chimeras
×
wild types
G1
wild types
Gr10⁴/-
other heterozygous
in-cross
G2
wild types
Gr10-/-
Gr10⁴/-
in-cross
G3
Gr10-/-
D
G0
Gr6 chimeras
×
wild types
G1
wild types
Gr6¹⁷/-
other heterozygous
in-cross
G2
wild types
Gr6-/-
Gr6¹⁷/-
in-cross
G3
Gr6-/-
B
Exon 1
sgRNA
5'
3'
PAM
TACACGGCTTTCCAAAAAGCGATGAAGGTTCT
Gr10
WT
TACACGGCTTTCCAAAAAGCGATGAAGGTTCT
Gr10-/-
-4bp
TACACGGCTTTCCAAAAA----TGAAGGTTCT
E
Exon1
sgRNA
5'
3'
PAM
ACCACCTGCGTGACGATGATGCTGTTCTTCCA
Gr6
WT
ACCACCTGCGTGACGATGATGCTGTTCTTCCA
Gr6-/-
-17bp
ACCACCTGCG----------TTCCA
C
Extracellular
Cytoplasm
WT
Gr10-/-
F
Extracellular
Cytoplasm
WT
Gr6-/-


A
Lateral sensilla styloconica in larvae
WT
Gr10-/-
Gr6-/-
KCl 2 mM
Suc 10 mM
Fuc 10 mM
Sin 10 mM
B
WT
Gr10-/-
Gr6-/-
Spikes/s
2 mM KCl
1 mM Suc
10 mM Suc
100 mM Suc
1 mM Fuc
10 mM Fuc
100 mM Fuc
10 mM Sin
C
Sensilla chaetica in adult antennae
KCl 2 mM
Suc 100 mM
Fuc 100 mM
Fru 100 mM
Nic 10 mM
D
2 mM KCl
10 mM Suc
100 mM Suc
10 mM Fuc
100 mM Fuc
100 mM Fru
10 mM Nic
E
Sensilla trichodea in adult tarsi
F
G
Sensilla styloconica in adult proboscis
H
2 mV
200 ms


A
10 mM Suc
Control
PI value
WT
Gr10-/-
Gr6-/-
**
ns
**
0.120 ± 0.059
0.013 ± 0.015
0.117 ± 0.035
1.2 0.8 0.4 0.0 0.4 0.8 1.2
Feeding area (cm2)
B
100 mM Suc
Control
PI value
WT
Gr10-/-
Gr6-/-
***
*
***
0.192 ± 0.044
0.100 ± 0.042
0.171 ± 0.046
1.2 0.8 0.4 0.0 0.4 0.8 1.2
Feeding area (cm2)
C
Feeding amount (g)
0.12
0.09
0.06
0.03
0.00
WT
Gr10-/-
Gr6-/-
Cabbage leaf
Corn
Pea
Pepper
Tomato
D
E
F
G
H
I
Percentage of PER
100
75
50
25
0
Sucrose (mM)
Fucose (mM)
Fructose (mM)
0 1 10 100 1000