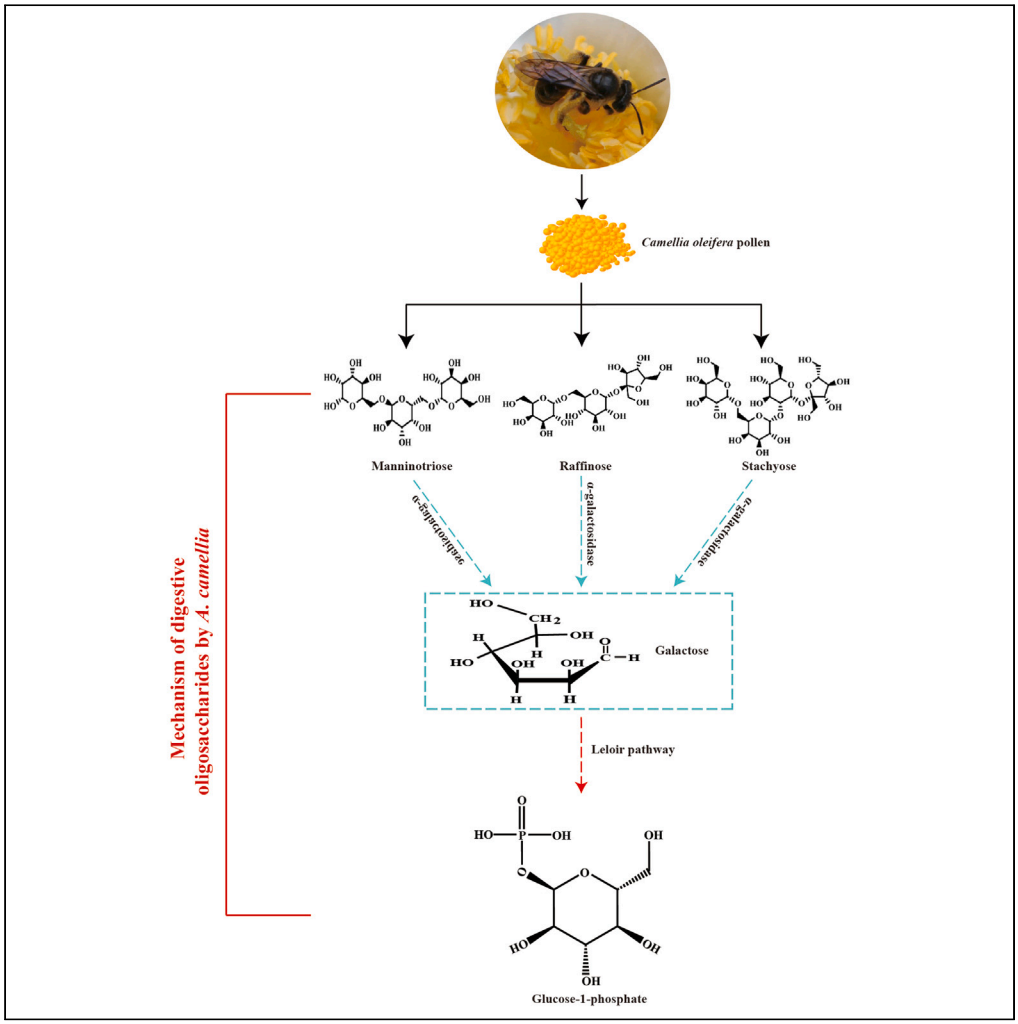
iScience

CellPress
OPEN ACCESS

Article

The mechanism of *Andrena camellia* in digesting toxic sugars



Zhen Li, Shiqing Zhong, Qiang Huang, ..., Wenkai Shi, Dongsheng Guo, Zhijiang Zeng

bees1965@sina.com

Highlights

The activity of GAL, GALT, GALK, and GALE in the gut of the *Andrena camellia* was strong

Identification of the metabolic profile of oligosaccharides in *Andrena camellia* gut

GAL, GALT, GALK, and GALE in the genome and were broadly conserved in *Andrena* spp

Li et al., iScience *27*, 109847
June 21, 2024
https://doi.org/10.1016/j.isci.2024.109847

iScience

CellPress
OPEN ACCESS

Article

The mechanism of *Andrena camellia* in digesting toxic sugars

Zhen Li,[1,2,3,5] Shiqing Zhong,[1,2,5] Qiang Huang,[1,2] Yong Zhang,[1,4] Tianyu Xu,[1,2] Wenkai Shi,[1,2] Dongsheng Guo,[3] and Zhijiang Zeng[1,2,6,*]

SUMMARY

Camellia oleifera is an economically and medicinally valuable oilseed crop. Honeybee, the most abundant pollinator, rarely visits _C. oleifera_ because of the toxic sugars in the nectar and pollen. These toxic sugars cannot be fully digested by honeybees and inhibit the process of synthesizing trehalose in honeybees. _C. oleifera_ exhibits self-incompatibility, and its pollination heavily depends on _Andrena camellia_. However, the mechanism by which _A. camellia_ digests toxic sugars in _C. oleifera_ nectar and pollen remains unknown. Consequently, we identified and validated four single-copy genes (_α-N-acetyl galactosamine-like_, _galactokinase_, _galactose-1-phosphate uridyltransferase_, and _UDP-galactose-4′-epimerase_, abbreviated as _NAGA-like_, _GALK_, _GALT_, and _GALE_) essential for detoxifying toxic sugars _in vitro_. Then, we cloned the four genes into _Escherichia coli_, and expressed enzyme successfully degraded the toxic sugars. The phylogeny suggests that the genes were conserved and functionally diverged among the evolution. These results provide novel insights into pollinator detoxification during co-evolution.

INTRODUCTION

Camellia oleifera is known for its high-quality oilseed, known as the Eastern olive oil.[1] It is a dominant oilseed crop cultivated in southern China.[2] *C. oleifera* exhibits self-incompatibility characteristics.[3,4] As a typical hetero-pollinated plant, *C. oleifera* uses pollinators to bear fruit.[5] Commercially reared honeybees with multiple combs are desirable pollinating insects and can be used to contribute to the efficient pollination of crops.[6–9]

However, when applying honeybees to pollinate *C. oleifera*, the honeybees are reluctant to visit *C. oleifera* flowers limiting the fruit yield because manninotriose, raffinose, and stachyose in nectar and pollen are toxic to bees.[10,11] The composition of manninotriose, raffinose, and stachyose in *C. oleifera* nectar were 0.25%, 2.88%, and 3.71%, respectively, while they were slightly higher, 0.30%, 3.40%, and 4.16% in *C. oleifera* pollen.[11] Thus, *C. oleifera* depends heavily on the wild pollinator *Andrena camellia*.[12,13] In the last two decades, *A. camellia* has declined sharply due to crop monoculture in *C. oleifera* forests, habitat destruction of *A. camellia*, intensification of anthropogenic activities, and abuse of pesticides (insecticides and herbicides).[11] *A. camellia* nests are declining dramatically.[10] Not all pollinators of the Apoidea are unsuitable for the toxic oligosaccharides in the nectar and pollen of *C. oleifera*, such as the bumblebee genus belonging to the Apoidea can visit *C. oleifera* flowers without being poisoned. But the application of bumblebees for pollination of *C. oleifera* cost too expensive.[14] Therefore, the dilemma of low efficiency of honeybee pollination for *C. oleifera* desperately needs to be busted.

A. camellia is a ground-nesting solitary bee belonging to the family Andrenidae.[15] Early in the blooming of *C. oleifera* flowers, *A. camellia* began to emerge from the nest room in the earth to collect nectar and pollen; the number of *A. camellia* peaked during the *C. oleifera* blooming period. The larvae and adult bees feed only on *C. oleifera* nectar and pollen throughout their lives.[16] *A. camellia* can digest toxic oligosaccharides in *C. oleifera* nectar and pollen. However, the mechanism by which *A. camellia* digests these oligosaccharides remains unclear. Mannitriose, raffinose, and stachyose all belong to α-galactosides, and their hydrolysis requires α-GAL enzyme catalysis.[17] The *NAGA-like* gene in the genome of the *Andrena* spp. plays the role of encoding the α-GAL enzyme.[18] In contrast, the breakdown of the three oligosaccharides into toxic galactose requires GALK, GALT, and GALE together.[19]

In this study, we revealed the mechanism adopted by *A. camellia* to digest toxic sugars using comparative genomics, metabolomics, prokaryotic protein expression, and *in vitro* digestion assays. The findings of this study provide insights into the functional co-evolution between *A. camellia* and *C. oleifera*.

[1]Honeybee Research Institute, Jiangxi Agricultural University, Nanchang 330045, China
[2]Jiangxi Province Key Laboratory of Honeybee Biology and Beekeeping, Nanchang 330045, China
[3]College of Life Science and Resources and Environment, Yichun University, Yichun 336000, China
[4]Jiujiang University, Jiujiang 332005, China
[5]These authors contributed equally
[6]Lead contact
*Correspondence: bees1965@sina.com
https://doi.org/10.1016/j.isci.2024.109847

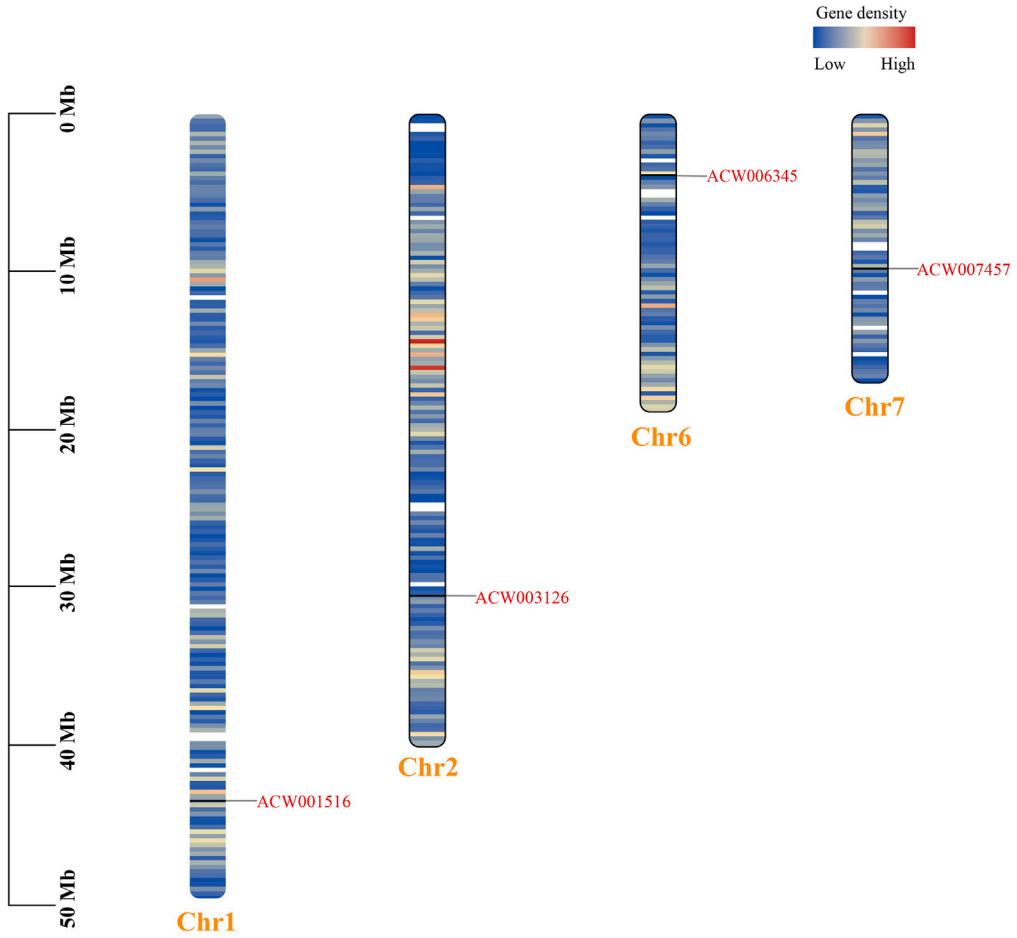


Figure 1. Chromosomal distribution of genes encoding GAL (ACW001516), GALK (ACW007457), GALT (ACW006345), and GALE (ACW003126) associated with oligosaccharide breakdown and galactose metabolism

RESULTS

Chromosomal locations of four genes that metabolize oligosaccharides in the genome of the A. camellia

In the genome of the *A. camellia*, we found that genes encoding galactosidase (*NAGA-like*), galactokinase (*GALK*), galactose 1-phosphate uridyltransferase (*GALT*), and uridine diphosphate-4′-epimerase (*GALE*) were located on chromosomes 1, 7, 6, and 2, respectively (Figure 1). The four genes were single copies in the genome and were broadly conserved in *Andrena* spp. (Table S1).

High gene expression profiles of NAGA-like, GALK, GALT, and GALE in *A. camellia*

The metabolism of manninotriose, raffinose, and stachyose to non-toxic UDP-glucose requires synergistic action of *NAGA-like*, *GALK*, *GALT*, and *GALE*. Therefore, we examined the expression levels of these four genes in the head and thorax of *A. camellia*, *A. cerana*, and *A. mellifera* after feed the stachyose. The relative expression levels of *NAGA-like*, *GALK*, and *GALT* genes in the head and thorax were significantly higher in *A. camellia* than in *A. cerana* and *A. mellifera* (*GAL*, $p < 0.001$; *GALK*, $p < 0.05$; *GALT*, $p < 0.001$) (Figures 2A–2C). High- and low-ranking GAL, GALK, and GALE enzyme activities in the guts of *A. camellia*, *A. cerana*, and *A. mellifera* were consistent with gene expression (GAL, $p < 0.001$; GALK, $p < 0.001$; GALT, $p < 0.001$) (Figures 2E–2G). The relative expression of the *GALE* gene in the head and thorax of *A. camellia* and *A. cerana* was significantly higher than that of *A. mellifera* (GALE, $p < 0.001$), and the difference between *A. camellia* and *A. cerana* was not significant ($p < 0.05$) (Figure 2D). GALE enzyme activity in the gut of *A. camellia* was significantly higher than that in the gut of *A. mellifera* ($p < 0.05$). The difference in GALE enzyme activity in the gut of *A. camellia* compared with that of *A. cerana* was not significant ($p > 0.05$), nor was the difference in GALE enzyme activity in the gut of *A. mellifera* compared with that of *A. cerana* (GALE, $p = 0.056$) (Figure 2H).

Clustering relationship analysis of NAGA-like, GALK, GALT, and GALE genes

Based on the four plots of the developmental tree, it can be understood that the *NAGA-like*, *GALT*, and *GALE* genes of the *A. camellia* clustered separately (Figures 3A, 3C, and 3D), in contrast, the *GALK* gene of the *A. camellia* clustered into a separate unit with *D. novaeangliae* (Figure 3B).

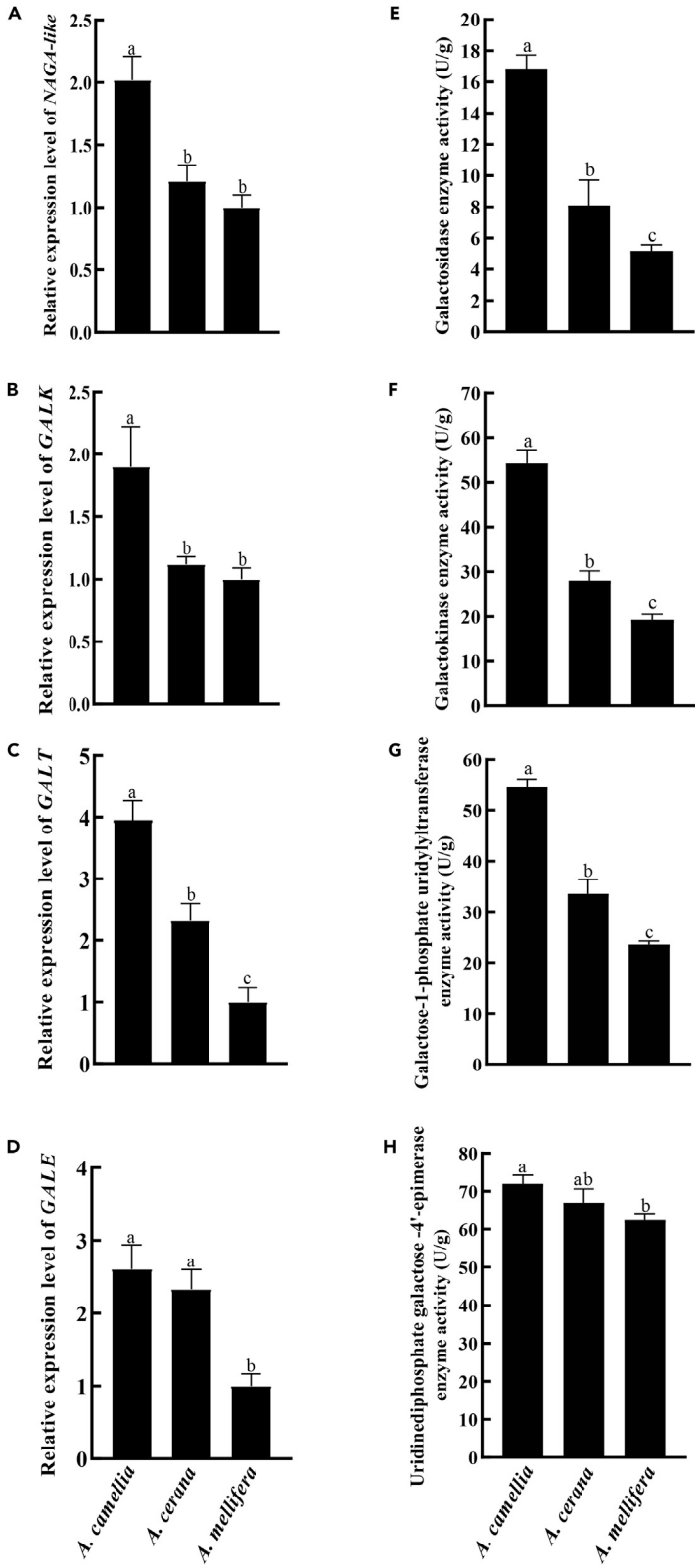

A
Relative expression level of NAGA-like
2.5
2.0
1.5
1.0
0.5
0.0
a
b
b
B
Relative expression level of GALK
2.5
2.0
1.5
1.0
0.5
0.0
a
b
b
C
Relative expression level of GALT
5
4
3
2
1
0
a
b
c
D
Relative expression level of GALE
4
3
2
1
0
a
a
b
A. camellia
A. cerana
A. mellifera
E
Galactosidase enzyme activity (U/g)
20
18
16
14
12
10
8
6
4
2
0
a
b
c
F
Galactokinase enzyme activity (U/g)
70
60
50
40
30
20
10
0
a
b
c
G
Galactose-1-phosphate uridylyltransferase enzyme activity (U/g)
60
50
40
30
20
10
0
a
b
c
H
Uridinediphosphate galactose -4'-epimerase enzyme activity (U/g)
80
70
60
50
40
30
20
10
0
a
ab
b
A. camellia
A. cerana
A. mellifera

Figure 2. The relative expression of *NAGA-like*, *GALK*, *GALT*, and *GALE* genes in the head and thorax of three honeybee species and GAL, GALK, GALT, and GALE enzymes in the gut of *A. camellia*, *A. cerana*, and *A. mellifera*, respectively (n=8)

(A) Relative expression of *NAGA-like* genes in the head and thorax of *A. camellia*, *A. cerana*, and *A. mellifera*.

(B) Relative expression of *GALK* genes in the head and thorax of *A. camellia*, *A. cerana*, and *A. mellifera*.

(C) Relative expression of the *GALT* genes in the head and thorax of *A. camellia*, *A. cerana*, and *A. mellifera*.

(D) Relative expression of *GALE* genes in the head and thorax of *A. camellia*, *A. cerana*, and *A. mellifera*.

(E) GAL enzyme activity in the guts of *A. camellia*, *A. cerana*, and *A. mellifera*.

(F) GALK enzyme activity in the guts of *A. camellia*, *A. cerana*, and *A. mellifera*.

(G) GALT enzyme activity in the guts of *A. camellia*, *A. cerana*, and *A. mellifera*.

(H) GALE enzyme activity in the guts of *A. camellia*, *A. cerana*, and *A. mellifera*. Note: The same lowercase letters in the same row indicate no significant differences ($p > 0.05$), and different lowercase letters indicate significant differences ($p < 0.05$).

Analysis of oligosaccharides and metabolite products in the gut of *A. camellia*, *A. cerana*, and *A. mellifera* by HPLC and LC-MS/MS

Manninotriose, raffinose, and stachyose accumulated in the gut of both *A. cerana* and *A. mellifera*, whereas *A. cerana* and *A. mellifera* broke down manninotriose, raffinose, and stachyose to form monosaccharide products including galactose, glucose, and fructose (Tables 1 and 2). *A. cerana* and *A. mellifera* produce intermediate melibiose after feeding on manninotriose, raffinose, or stachyose (Table 1). However, *A. camellia* effectively decomposed oligosaccharides and produced galactose (Table 2). More importantly, *A. camellia* could metabolize toxic galactose to non-toxic UDP-glucose, whereas *A. cerana* and *A. mellifera* could not effectively digest galactose (Table 2).

Hydrolysis of oligosaccharides and galactose using GAL, GALK, GALT, and GALE enzymes *in vitro*

We purified and expressed the four enzymes (Figure S3) by transferring plasmids with GAL, GALK, GALT, and GALE coding region sequences (from the *A. camellia* genome) into *E. coli*. The *in vitro* digestion results showed that mannotriose, raffinose, and stachyose could be hydrolyzed by GAL, whereas galactose, the basic structure of the three oligosaccharides, was converted to 1-phosphate, UDP-galactose, and UDP-glucose by GALK, GALT, and GALE.

After the addition of GAL, the mannanotriose, raffinose, and stachyose contents significantly decreased ($p < 0.05$) in the treatment group compared with those in the control group, and the hydrolysis efficiencies reached 6.63 $\pm$ 1.18%, 12.32 $\pm$ 0.35%, and 15.13 $\pm$ 4.34%, respectively (Figures 4A–4C). Compared with the control group, the sequential addition of GALK, GALT, and GALE to the galactose mother liquor resulted in a significant decrease ($p < 0.05$) in the galactose content of the treated group, with a galactose of 26.39 $\pm$ 0.35% (Figure 4D). Moreover, three galactose metabolites, including galactose 1-phosphate, UDP-galactose and UDP-glucose, which were not found in the control group, were detected in treated groups (Figures 4E–4G).

DISCUSSION

Mannotriose is a trisaccharide comprising two galactose molecules and one glucose molecule. Two galactose molecules are linked to one glucose molecule on both sides via two α-(1→6) galactosidic bonds.[11] The hydrolysis of an α-(1→6) galactosidic bond by GAL produces one molecule each of galactose and one molecule of melibiose. It explains why the intermediate product melibiose was detected in the gut of *A. camellia* and *A. mellifera* after feeding on mannitritose, raffinose, or stachyose. Raffinose, a member of the raffinose family of oligosaccharides (RFOs), is composed of sucrose and chains of α-galactosyl residues attached to the glucose moiety of sucrose via an α-(1→6) galactosidic linkage.[20] The stachyose composition is "galactose-galactose-glucose-fructose"; therefore, it can be considered as one molecule of galactose linked to one molecule of raffinose, or as one molecule of fructose bound to one molecule of mannotriose.[11]

This study showed that raffinose and stachyose do not accumulate over time when *A. camellia* feeds on them because the gut secretes more active GAL to hydrolyze raffinose and stachyose to release galactose. Although mannotriose accumulates over time in the gut of *A. camellia*, the low accumulation in the gut compared with *A. cerana* and *A. mellifera* may be due to the low mannotriose content added in the diet (0.30%), and the dynamic equilibrium of mannotriose degradation by *A. camellia* up to a certain safe concentration. When *A. camellia* was fed stachyose, the intermediate products, raffinose and mannotriose, were detected in their guts, as in the case of *A. cerana* and *A. mellifera*. This is because α-galactosidase breaks the α-(1→6) galactosidic linkage on one side to release raffinose, or β-fructosidase hydrolyzes the α-(1→2) glycosidic bond on the other side to release mannotriose. Additionally, the raffinose content in the gut of *A. camellia* was higher than that of mannotriose at 48th h and 72 nd h after feeding with stachyose (Table 2). It was also confirmed that the GAL enzyme activity was higher in the gut of *A. camellia*. Indeed, we speculated that the gene encoding GAL in the genome of the *A. camellia* is *α-N-acetyl galactosamine-like* (*NAGA-like*). Molecular evolutionary analyses using branching and branching site models showed that the NAGA-like gene was under strong positive selection in the genomes of seven *Andrena* species, including the *A. camellia*, indicating that new phenotypes for this gene will emerge in these species.[18]

Galactose produced by hydrolysis of mannitritose, raffinose, and stachyose is toxic to Apidae, with a tolerance of no more than 5%.[21] However, our results indicated that *A. camellia* can metabolize oligosaccharides to produce UDP-glucose through the secretion of highly active GAL, GALK, GALT, and GALE enzyme. Furthermore, UDP-glucose undergoes glucose phosphorylase and glucose-6-phosphate translocase

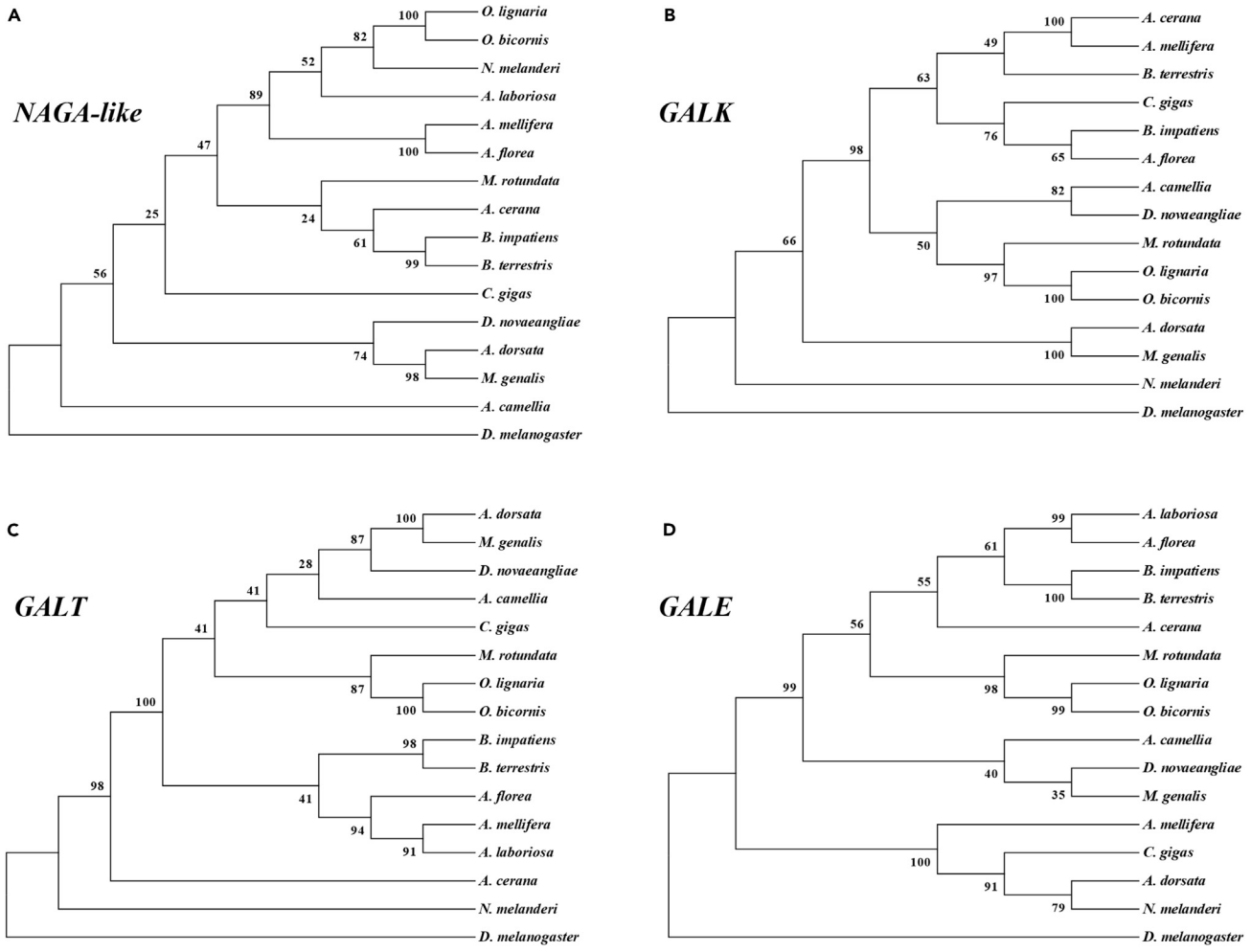


Figure 3. Clustering relationship analysis of *NAGA-like*, *GALK*, *GALT*, and *GALE* in fifteen honeybee genomes, with Drosophila melanogaster as the outgroup

(A) Clustering relationship analysis of *NAGA-like* gene in 16 species.
(B) Clustering relationship analysis of *GALK* gene in 16 species.
(C) Clustering relationship analysis of *GALT* gene in 16 species.
(D) Clustering relationship analysis of *GALE* gene in 16 species.

activity to generate glucose-6-phosphate for entry into the pentose phosphate and glycolytic pathways.[19] The high activity of GALK, GALT, and GALE enzymes in the gut of *A. camellia* was also convincing evidence that galactose is digested by these enzymes.

In summary, the decomposition of oligosaccharides in *A. camellia* was divided into two steps. Galactosidase breaks down the three macromolecular sugars into small molecules of glucose, fructose, and galactose (Figure S4). Galactose then passes through "The Leloir Pathway" to form UDP-glucose, which is subsequently phosphorylated and enters the glycolytic pathway[11,22] (Figure S4). Most importantly, in the second step, GAL, GALK, GALT, and GALE enzyme must catalyze successive steps to complete the process.

These findings provide insights into the mutually beneficial symbiotic mechanisms of *A. camellia* and *C. oleifera*. As for the lethal mechanism of *A. cerana* and *A. mellifera* after foraging on mannitritose, raffinose, and stachyose, there are 2 points: (1) *A. cerana* and *A. mellifera* are unable to digest the three oligosaccharides adequately, which can cause the accumulation of oligosaccharides in the gut; (2) *A. cerana* and *A. mellifera* are also less able to digest the toxic galactose produced by digestion of oligosaccharides.

Limitations of the study

Although this study showed that the sequences of the coding regions of four enzymes in the *A. camellia* were introduced into *E. coli* and expressed *in vivo* to produce enzymes that could digest oligosaccharides and galactose that are toxic to honeybees in *in vitro* experiments, the mechanism of their functioning in the *A. camellia* and honeybee is not yet fully elucidated. The mechanism of digestion of

Table 1. Compositions of manninotriose, raffinose, and stachyose and their metabolites in gut of *Andrena camellia*, *Apis cerana*, and *Apis mellifera* at different time points (n = 3)

Saccharine	Honeybee species	Time points (h)	Absolute composition, μg/mg								
			Trehalose	Glucose	Fructose	Sucrose	Melibiose	Manninotriose	Lactose	Raffinose	Stachyose
Manninotriose	*A. camellia*	48	2.53 ± 0.05b	3.09 ± 0.15a	6.37 ± 0.11b	ND	ND	0.22 ± 0.004e	ND	ND	ND
		72	4.49 ± 0.12a	5.89 ± 0.28b	48.14 ± 1.22a	ND	ND	1.51 ± 0.06d	ND	ND	ND
	A. cerana	48	0.82 ± 0.23d	0.19 ± 0.04d	3.16 ± 0.80c	ND	0.25 ± 0.02b	7.96 ± 1.76b	ND	ND	ND
		72	0.84 ± 0.05d	0.26 ± 0.02c	2.86 ± 1.00c	ND	0.49 ± 0.09a	13.69 ± 0.66a	ND	ND	ND
	A. mellifera	48	1.37 ± 0.13c	0.19 ± 0.03d	3.45 ± 0.27c	ND	0.36 ± 0.04b	6.91 ± 1.03c	ND	ND	ND
		72	1.38 ± 0.09c	0.18 ± 0.02d	3.64 ± 0.29c	ND	0.41 ± 0.05b	9.54 ± 1.08c	ND	ND	ND
Raffinose	*A. camellia*	48	5.51 ± 0.29a	3.87 ± 0.17b	80.35 ± 4.17a	ND	ND	ND	ND	6.99 ± 0.46c	ND
		72	4.72 ± 0.12b	6.08 ± 0.26a	80.99 ± 1.70a	ND	ND	ND	ND	7.04 ± 0.14c	ND
	A. cerana	48	1.11 ± 0.09c	0.32 ± 0.04c	7.77 ± 1.15b	ND	14.71 ± 0.51b	ND	ND	6.43 ± 0.82c	ND
		72	1.29 ± 0.17c	0.21 ± 0.05cd	8.41 ± 0.74b	ND	15.03 ± 0.13b	ND	ND	13.92 ± 0.69a	ND
	A. mellifera	48	1.14 ± 0.10c	0.15 ± 0.02d	2.56 ± 0.34c	ND	12.69 ± 0.67c	ND	ND	7.76 ± 0.84c	ND
		72	1.28 ± 0.10c	0.12 ± 0.01d	5.51 ± 0.67c	ND	19.57 ± 1.56a	ND	ND	11.32 ± 1.11b	ND
Stachyose	*A. camellia*	48	3.61 ± 0.09b	4.59 ± 0.25b	96.87 ± 2.51a	ND	ND	0.25 ± 0.01c	ND	1.58 ± 0.04b	13.02 ± 0.32b
		72	4.27 ± 0.04a	6.39 ± 0.27a	86.15 ± 0.97b	ND	ND	0.44 ± 0.02c	ND	1.85 ± 0.04a	9.13 ± 0.11c
	A. cerana	48	1.08 ± 0.05cd	0.23 ± 0.01c	7.78 ± 1.58c	ND	1.07 ± 0.11ab	24.87 ± 1.31b	ND	0.06 ± 0.004d	9.59 ± 0.55c
		72	1.18 ± 0.14c	0.21 ± 0.03cd	10.79 ± 0.88c	ND	1.23 ± 0.08a	27.19 ± 1.15a	ND	0.14 ± 0.01c	28.65 ± 1.57a
	A. mellifera	48	1.07 ± 0.20cd	0.15 ± 0.01de	3.76 ± 0.44e	ND	1.00 ± 0.15ab	25.78 ± 2.77a	ND	0.055 ± 0.01day	8.10 ± 0.40c
		72	0.76 ± 0.03d	0.12 ± 0.02e	5.08 ± 0.47e	ND	1.39 ± 0.07b	34.66 ± 3.32a	ND	0.14 ± 0.01c	19.83 ± 0.70b

Abbreviation: ND, no detected.

The same lowercase letters in the same column indicate no significant difference ($p > 0.05$), different lowercase letters indicate significant difference ($p < 0.05$). The same as the following table.

Table 2. Composition of galactose and its metabolites in the gut of *A. camellia*, *A. cerana*, and *A. mellifera* after foraging for mannitritose, raffinose, and stachyose at different time points (*n* = 3)

			Absolute composition, μg/g			
Saccharine	Honeybee species	Time points (h)	Galactose	Galactose 1-phosphate	UDP-galactose	UDP-glucose
Manninotriose	*A. camellia*	48	148.89 ± 0.88a	13.06 ± 0.40b	7.73 ± 0.26b	0.03 ± 0.001c
		72	154.34 ± 7.27a	14.61 ± 0.516a	9.92 ± 1.02a	0.04 ± 0.001c
	A. cerana	48	14.33 ± 2.06b	1.21 ± 0.22c	0.90 ± 0.10c	0.08 ± 0.01b
		72	18.71 ± 4.64b	1.25 ± 0.02c	0.93 ± 0.10c	0.07 ± 0.01b
	A. mellifera	48	17.16 ± 4.57b	1.17 ± 0.02c	0.95 ± 0.01c	0.09 ± 0.001ab
		72	18.45 ± 1.97b	1.43 ± 0.06c	0.88 ± 0.08c	0.10 ± 0.002a
Raffinose	*A. camellia*	48	412.15 ± 20.86b	14.70 ± 0.42b	9.93 ± 0.65a	0.03 ± 0.001a
		72	552.70 ± 15.72a	15.60 ± 0.41a	10.56 ± 0.34a	0.04 ± 0.001a
	A. cerana	48	149.46 ± 6.69d	1.39 ± 0.08c	0.99 ± 0.02b	0.07 ± 0.0052b
		72	276.32 ± 36.22c	1.59 ± 0.10c	0.88 ± 0.16b	0.06 ± 0.005b
	A. mellifera	48	72.55 ± 12.60e	1.23 ± 0.05c	0.78 ± 0.04b	0.08 ± 0.005b
		72	102.92 ± 11.43d	1.56 ± 0.08c	0.76 ± 0.03b	0.09 ± 0.009b
Stachyose	*A. camellia*	48	298.43 ± 11.60b	13.81 ± 0.30b	9.40 ± 0.21b	0.04 ± 0.001c
		72	686.04 ± 15.82a	16.19 ± 0.36a	16.59 ± 1.75a	0.04 ± 0.001c
	A. cerana	48	189.59 ± 4.97c	1.48 ± 0.03b	0.90 ± 0.11c	0.08 ± 0.006b
		72	268.33 ± 12.56b	1.69 ± 0.08b	1.29 ± 0.21c	0.08 ± 0.02b
	A. mellifera	48	55.65 ± 3.48d	1.46 ± 0.22b	0.94 ± 0.16c	0.13 ± 0.04a
		72	75.58 ± 4.29d	1.86 ± 0.14b	1.20 ± 0.25c	0.15 ± 0.03a

oligosaccharides and galactose in the *A. camellia* in *in vivo* experiments is not well characterized and may require isotopic tracing to be fully elucidated.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- RESOURCE AVAILABILITY
  - Lead contact
  - Materials availability
  - Data and code availability
- METHOD DETAILS
  - Insects collection
  - Enzyme activity of GAL, GALK, GALT and GALE in the gut of *A. camellia*, *A cerana* and *A. mellifera*
  - Sample treatment and analysis of oligosaccharides and breakdown products in the gut of three types of bees by high-performance liquid chromatography (HPLC)
  - The relative expression level of four genes in the heads and thorax of *A. camellia*, *A. cerana*, and *A. mellifera*
  - Clustering relationship analysis of NAGA-like, GALK, GALT, and GALE genes in different honey bee genomes
  - *In vitro* expression of four enzymes and *in vitro* digestion of three oligosaccharides and galactose
  - Analysis the efficiency of hydrolysis with three types of oligosaccharides with GAL *in vitro* by HPLC
  - Data access
- QUANTIFICATION AND STATISTICAL ANALYSIS
  - Data analysis

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2024.109847.

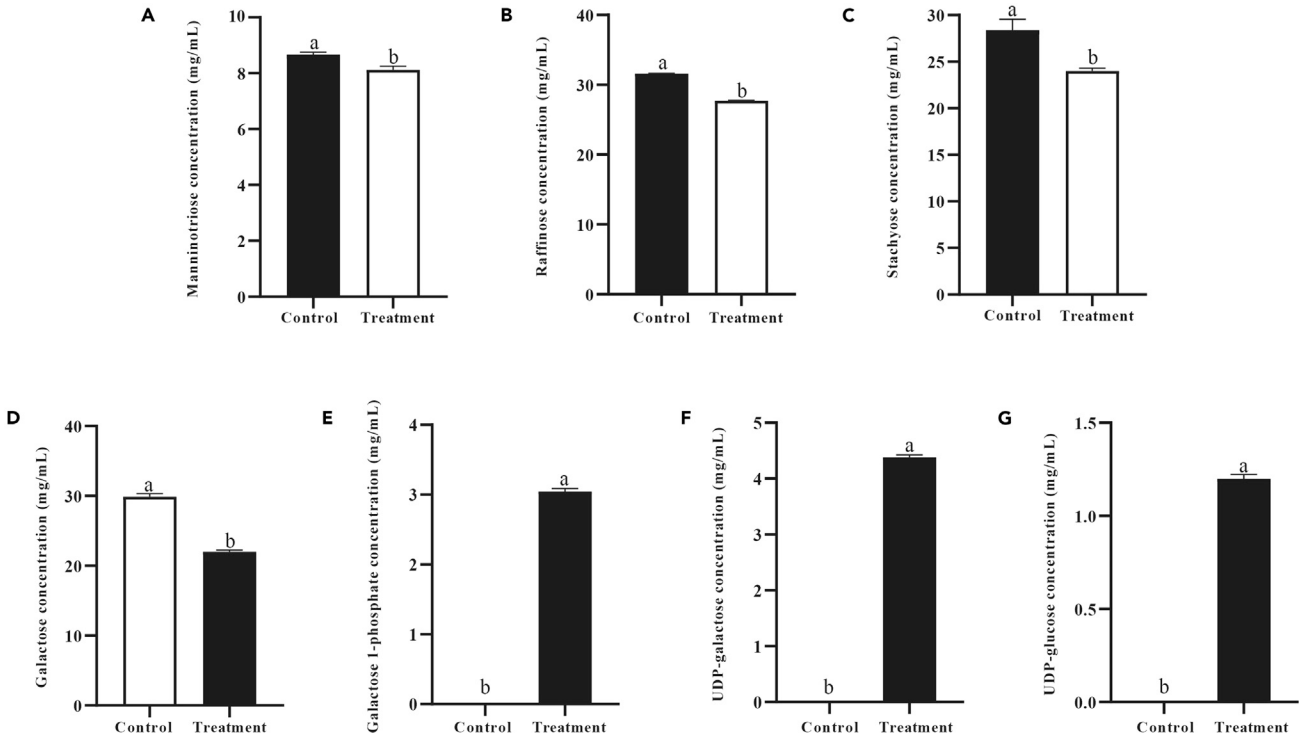


Figure 4. Residual sugar levels after 48 h of continuous digestion of manninotriose, raffinose, and stachyose with the addition of GAL and GALK, GALT and GALE enzyme generate galactose 1-phosphate, UDP-galactose and UDP-glucose levels upon 48 h of continuous digestion of galactose *in vitro*

(A) Manninotriose concentrations in the control and treatment groups.

(B) Raffinose concentrations in the control and treatment groups.

(C) Stachyose concentrations in control and treatment groups.

(D) Galactose concentrations in the control and treatment groups.

(E) Galactose 1-phosphate concentrations in the control and treatment groups.

(F) UDP-galactose concentrations in the control and treatment groups.

(G) UDP-glucose concentrations in control and treatment groups.

ACKNOWLEDGMENTS

This work was supported by the Earmarked Fund for the China Agricultural Research System (CARS-44-KXJ15) and the National Natural Science Foundation of China (32172790).

AUTHOR CONTRIBUTIONS

Z.L. and Z.J.Z. designed research; Z.L., S.Q.Z., and T.Y.X. performed research; Z.J.Z., Q.H., and D.S.G. provided guidance for data; Z.L., Y.Z., and W.K.S. analyzed data; Z.L. and Z.J.Z. wrote the paper.

DECLARATION OF INTERESTS

The authors declare no conflicts of interests.

Received: February 14, 2024
Revised: April 10, 2024
Accepted: April 25, 2024
Published: April 29, 2024

REFERENCES

1. Feng, J.L., Yang, Z.J., Chen, S.P., El-Kassaby, Y.A., and Chen, H. (2017). High throughput sequencing of small RNAs reveals dynamic microRNAs expression of lipid metabolism during *Camellia oleifera* and *C. meiocarpa* seed natural drying. BMC Genom. *18*, 546.

2. Lin, P., Wang, K., Wang, Y., Hu, Z., Yan, C., Huang, H., Ma, X., Cao, Y., Long, W., Liu, W., et al. (2022). The genome of oil-Camellia and population genomics analysis provide

insights into seed oil domestication. Genome Biol. *23*, 14–21.

3. Zhang, X.F., Yang, S.L., Han, Y.Y., Zhao, L., Lu, G.L., Xia, T., and Gao, L.P. (2014). Qualitative and quantitative analysis of triterpene saponins from tea seed pomace (*Camellia oleifera* abel) and their activities against bacteria and fungi. Molecules *19*, 7568–7580.
4. Lu, M., Zhou, J., Liu, Y., Yang, J., and Tan, X. (2021). CoNPR1 and CoNPR3. 1 are involved in SA-and MeSA-mediated growth of the pollen tube in *Camellia oleifera*. Physiol. Plantarum *172*, 2181–2190.
5. Aizen, M.A., Aguiar, S., Biesmeijer, J.C., Garibaldi, L.A., Inouye, D.W., Jung, C., Martins, D.J., Medel, R., Morales, C.L., Ngo, H., et al. (2019). Global agricultural productivity is threatened by increasing pollinator dependence without a parallel increase in crop diversification. Global Change Biol. *25*, 3516–3527.
6. Cecala, J.M., Lau, P.W., and Leong, J.M. (2020). Floral bagging differentially affects handling behaviours and single-visit pollen deposition by honey bees and native bees. Ecol. Entomol. *45*, 1099–1107.
7. Milner, J.R.D., Bloom, E.H., Crowder, D.W., and Northfield, T.D. (2020). Plant evolution can mediate negative effects from honey bees on wild pollinators. Ecol. Evol. *10*, 4407–4418.
8. Subasinghe Arachchige, E.C.W., Rader, R., Cutting, B.T., Keir, M., van Noort, T., Fale, G., Howlett, B.G., Samnegård, U., and Evans, L.J. (2022). Honey bees are the most abundant visitors to Australian watermelon but native stingless bees are equally effective as pollinators. Ecol. Solut. Evid. *3*, e12189.
9. Qiu, L., Dong, J., Li, X., Parey, S.H., Tan, K., Orr, M., Majeed, A., Zhang, X., Luo, S., Zhou, X., et al. (2023). Defining honeybee subspecies in an evolutionary context warrants strategized conservation. Zool. Res. *44*, 483–493.
10. Xie, Z., Chen, X., and Qiu, J. (2013). Reproductive failure of camellia oleifera in the plateau region of China due to a shortage of legitimate pollinators. Int. J. Agric. Biol. *15*.
11. Li, Z., Huang, Q., Zheng, Y., Zhang, Y., Li, X., Zhong, S., and Zeng, Z. (2022). Identification of the toxic compounds in *Camellia oleifera* honey and pollen to honey bees (*Apis mellifera*). J. Agric. Food Chem. *70*, 13176–13185.
12. Li, H., Orr, M.C., Luo, A., Dou, F., Kou, R., Hu, F., Zhu, C., and Huang, D. (2021). Relationships between wild bee abundance and fruit set of *Camellia oleifera* Abel. J. Appl. Entomol. *145*, 277–285.
13. Su, T., He, B., Zhao, F., Jiang, K., Lin, G., and Huang, Z. (2022). Population genomics and phylogeography of *Colletes gigas*, a wild bee specialized on winter flowering plants. Ecol. Evol. *12*, e8863.
14. Zhao, B., Xu, G., X, Z., Li, Q., Yi, Y., Yao, F., Shi, X., and Li, M. (2018). First trials to pollinate Camellia oleitera by using Bombus terrestris. J. Nanjing For. Univ. (Nat. Sci. Ed.) *61*, 175.
15. Pisanty, G., Richter, R., Martin, T., Dettman, J., and Cardinal, S. (2022). Molecular phylogeny, historical biogeography and revised classification of andrenine bees (Hymenoptera: Andrenidae). Mol. Phylogenet. Evol. *170*, 107151.
16. Huang, D., Kou, R., Orr, M.C., Li, H., Dou, F., and Zhu, C. (2021). Comparison of two criteria on the essential number calculation of *Andrena camellia*. Bull. Entomol. Res. *111*, 364–370.
17. Zhang, J., Song, G., Mei, Y., Li, R., Zhang, H., and Liu, Y. (2019). Present status on removal of raffinose family oligosaccharides-a review. Czech J. Food Sci. *37*, 141–154.
18. Lin, G., Huang, Z., He, B., Jiang, K., Su, T., and Zhao, F. (2023). Evolutionary adaptation of genes involved in galactose derivatives metabolism in oil-tea specialized Andrena species. Genes *14*, 1117.
19. Daenzer, J.M.I., Sanders, R.D., Hang, D., and Fridovich-Keil, J.L. (2012). UDP-galactose 4′-epimerase activities toward UDP-Gal and UDP-GalNAc play different roles in the development of *Drosophila melanogaster*. PLoS Genet. *8*, e1002721.
20. Wakabayashi, T., Joseph, B., Yasumoto, S., Akashi, T., Aoki, T., Harada, K., Muranaka, S., Bamba, T., Fukusaki, E., Takeuchi, Y., et al. (2015). Planteose as a storage carbohydrate required for early stage of germination of Orobanche minor and its metabolism as a possible target for selective control. J. Exp. Bot. *66*, 3085–3097.
21. Peng, Y.S. (1981). Tolerance of lactose by free-flying adult worker honeybees. J. Apicult. Res. *20*, 89–93.
22. Tang, M., Etokidem, E., and Lai, K. (2016). The Leloir pathway of galactose metabolism–a novel therapeutic target for hepatocellular carcinoma. Anticancer Res. *36*, 6265–6271.
23. Zheng, H., Perreau, J., Powell, J.E., Han, B., Zhang, Z., Kwong, W.K., Tringe, S.G., and Moran, N.A. (2019). Division of labor in honey bee gut microbiota for plant polysaccharide digestion. Proc. Natl. Acad. Sci. USA *116*, 25909–25916.
24. Livak, K.J., and Schmittgen, T.D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods *25*, 402–408.

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Biological samples		
Andrena camellia, *Apis cerana* and *Apis mellifera*	This paper	N/A
Critical commercial assays		
GAL Reagent Kit	Mlbio (China)	Cat#: YJ103503
GALK Reagent Kit	Mlbio (China)	Cat#: YJ104005
GALT Reagent Kit	Mlbio (China)	Cat#: YJ102042
GALE Reagent Kit	Mlbio (China)	Cat#: YJ1050471
TRIzol reagent (For RT-qPCR)	Tiangen (China)	Cat#: DP424
TB Green ® Premix ExTaqTM II	Takara (Japan)	Cat#: DRR081A
TB Green® Premix Ex Taq™ II (Tli RNaseH Plus), ROX plus	Takara (Japan)	Cat#: RR82LR
Prime *of NAGA-like*	Sangon Biotech (China)	N/A
Prime *of GALK*	Sangon Biotech (China)	N/A
Prime of *GALT*	Sangon Biotech (China)	N/A
Prime of *GALE*	Sangon Biotech (China)	N/A
Mannotriose	Sigma (USA)	Cat#: M2800-10UG
Raffinose	Sigma (USA)	Cat#: Sigma R0250
Stacyose	Sigma (USA)	Cat#: S4001-100MG
DNA Gel Recovery Kit	Dongsheng Biotech (China)	Cat#: CB84698694
XhoI/BamHI	Takara (Japan)	Cat#: 1094S
T4 DNA Ligase	Takara (Japan)	Cat#: 2011B
Deposited data		
Andrena camellia genome at the chromosomal level	NCBI database	BioProject: PRJNA921724
Experimental models: Organisms/strains		
Andrena camellia, *Apis cerana* and *Apis mellifera*	Xicun Town, Yuanzhou District, Yichun City, Jiangxi Province, China	N/A
Software and algorithms		
MEGA	www.megasoftware.net	version 7.0
IBM SPSS Statistics	SPSS institute Inc	version 25.0
PrimerPremier 5	Premier (Canada)	version 5.0

RESOURCE AVAILABILITY

Lead contact

For information and requests for resources, please contact Zhi Jiang Zeng (bees1965@sina.com).

Materials availability

This study did not generate new unique reagents.

Data and code availability

A. camellia genome at the chromosomal level have been deposited in NCBI under accession number PRJNA921724. All other data are included in the supplemental information.

METHOD DETAILS

Insects collection

The samples of wild female *A. camellia* were collected from the *C. oleifera* plantation in Xicun Town, Yuanzhou District, Yichun City (114°20′ N, 27°73′ E). *Apis cerana* and *Apis mellifera* were placed in the same *C. oleifera* plantation, with five experimental colonies of each bee species.

Enzyme activity of GAL, GALK, GALT and GALE in the gut of *A. camellia, A cerana* and *A. mellifera*

The gut of the foragers from *A. camellia*, *A. cerana*, or *A. mellifera* was dissected (discarding the honey sacs, 5 bees' guts made up one sample, 8 biological replicates for each species) in 1.5 mL sterile EP tubes, 1 000 μL ultrapure water was added and well grinded. The supernatant was collected after 5 min of standing, centrifuged at 12 000 rpm for 10 min and assessed for the enzyme activity of galactosidase (GAL), galactokinase (GALK), galactose-1-phosphate uridyltransferase (GALT) and UDP-galactose-4-epimerase (GALE) by ELISA (Mlbio Biotechnology Co., Shanghai, China) according to per the manufacturer's instructions.

Sample treatment and analysis of oligosaccharides and breakdown products in the gut of three types of bees by high-performance liquid chromatography (HPLC)

Before dietary treatment, bees (*A. camellia*, *A. cerana*, and *A. mellifera* foragers) were starved for 2 h and then fed mannotriose, raffinose, or stachyosedissolved in 50% (w/w) fructose solution. The three oligosaccharides were added in the same amount as in the *C. oleifera* pollen. After 48 h and 72 h of treatment, the entire gut was dissected from. Three oligosaccharide solutions were prepared by dissolving mannitritose, raffinose and stachyose (Sigma, Missouri, US, LC-MS, >99% pure) in 50% fructose solution according to the mass ratio (w/w) in *C. oleifera* pollen, individually.[11] For detailed diet ratios, describe as Table S2. Collecting foragers of *A. camellia*, *A. cerana* and *A. mellifera*, respectively. All three types of bees were divided into three treatment groups, and each of the three groups was adequate supplied with a 50% fructose solution containing three individual oligosaccharide diets. Each treatment group was set up in three biological replicates, comprising 30 honey bees per replicate (reared in a plastic cup cage). The plastic cup cages with *A. cerana* and *A. mellifera* were placed in an incubator at a temperature of 34.5°C and a relative humidity of 75%. The cup cages with *A. camellia* were incubated at a temperature of 25°C and 45% relative humidity. At the beginning of the individual oligosaccharide feeding treatment, all honey bees were starved for 2 h.[23] The gut from the three treatment groups of the three honey bees (with the honey sacs removed) was dissected in 1.5 mL sterile EP tubes at 48th h and 72nd h after the start of feeding, separately. The gut of 15 honey bees from each cup cage was dissected each time to comprise one sample, and three samples were taken from each treatment group each time. Before dissection, the 15 honey bees in each group were weighed, and the gut dissected out of each tube was also weighed for subsequent weight conversions. Meanwhile, the foraging amounts of the three types of honey bees at the 48th h and 72nd h were also weighed and recorded.

After weighing 15 kinds of saccharide standards separately and accurately (see Table S3 for 15 saccharide standards information), add ultrapure water to prepare 10 mg/mL single standard master batch, and then take the appropriate amount of single standard master batch to prepare 40 μg/mL standard mixed standard (for 15 types of saccharide concentration, refer to Table S4). Accurately weigh 50 mg of freeze-dried gut powder sample (three samples per treatment group) in a 2.0 mL centrifuge tube and add 700 μL of 80% ethanol. The sample was shaken at 50°C for 2 h and then diluted with 700 μL H_2O and centrifuged at 10 000 rpm for 3 min. Lastly, the supernatant was transferred to a 1.5 mL injection vial.

A Thermo ICS5000 ion chromatograph (Thermo Fisher Technology Co., Ltd., Waltham, MA, USA) equipped with an electrochemical detector and a CarboPac™ PA1 (250×4.0 mm, 4 μm, Thermo Fisher Technology, Waltham, MA, USA) chromatographic column was used to construct standard curves for 15 saccharides (working solution concentration as the x-axis and peak area as the y-axis) and to simultaneously perform absolute quantification of saccharides in honey bee gut samples. The mobile phases were A: H_2O, B: 100 mM NaOH; the injection volume was 10 μL, the flow rate was 1.0 mL/min, and the column temperature was 30°C. Elution gradient: 0.0∼12.0 min, 5% ∼10%B; 12.1∼15.0 min, 5%∼100% B; 15.1∼25.0 min, 100% B; 25.1∼40.0 min, 100%∼5% B; 40.1∼60.0 min, 5% B. Fitting information for the 15 saccharides was presented in Table S5.

Appropriate amounts of galactose 1-phosphate, uridine diphosphate galactose (UDP-galactose) and uridine diphosphate glucose (UDP-glucose) standards (Sigma, Missouri, US, LC-MS, > 99% pure) were accurately weighed and prepared into a single standard master batch of 10 mg/mL with methanol, individually. This was followed by aspirating three appropriate amounts of the saccharide single standard master batch to compose mixed standard solutions of different concentrations as shown in Table S6.

50 mg of freeze-dried *A. camellia*, *A. cerana* or *A. mellifera* gut sample (three samples per treatment group, all honey bees were foragers) was weighed into a 5 mL sterile centrifuge tube and 1000 μL of extraction solution (methanol: acetonitrile: water = 2:2:1, v/v) was added. Ultrasonic treatment in the ice water bath for 10 min, liquid nitrogen flash freezing for 1 min, repeated three times. Samples were placed at -20°C for 1 h and later centrifuged at 13 000 rpm for 15 min at 4°C. The supernatant was removed by aspirating 800 mL into a new 2 mL sterile centrifuge tube, blowing dry with a nitrogen blower and adding 600 μL of 50% acetonitrile aqueous solution (v/v) for re-dissolution. Dissolved samples were shaken for 30 s and then sonicated in an ice water bath for 10 min. Following sonication, the samples were centrifuged at 3 000 rpm for 15 min at 4°C and 400 μL of supernatant was injected into a 1.5 mL injection vial.

High-performance liquid chromatography (LC-30A, Shimaduzu Co. Tokyo, Japan) tandem mass spectrometry (AB Sciex, 5600 Q-TOF/6500 Q-TRAP, Framingham, MA, USA) was applied to construct standard curves for the three saccharides (working solution concentration as the x-axis and peak area as the y-axis) and to concurrently detect the absolute compositions of galactose 1-phosphate, UDP-galactose

and UDP-glucose in all honey bee gut samples. Separations were carried out using an ACQUITY UPLC® BEH C18 chromatographic column (2.1×100 mm, 1.7 μm, Waters, Milford, MA, USA). Analytes were separated by ultrapure water (containing 25 mM CH_3COONH_4 and 25 mM NH_4OH) (A) and Acetonitrile (B) at a flow-rate of 0.3 mL/min. The linear gradient elution program was: 0.0∼1.0 min, 85% B; 1.1∼12.0 min, 65% B; 12.1∼15.0 min, 40% B; 15.1∼20.0 min, 85% B. The column was thermostated at 40°C and injection volume 5 μL.

Mass spectrometry conditions: electrospray ionization source (ESI). Ion-source temperature 600°C, ion-source voltage 5500 V, curtain gas 20 psi, nebulizer gas and auxiliary gas 60 psi. Scanning was performed using multiple reaction monitoring (MRM).

The standard curves for the galactose 1-phosphate, uridine diphosphate galactose and uridine diphosphate glucose were provided in Table S7.

The relative expression level of four genes in the heads and thorax of *A. camellia*, *A. cerana*, and *A. mellifera*

To explore the *NAGA-like*, *GALK*, *GALT*, and *GALE* genes expression of the head and thorax of the *A. camellia*, *A. cerana* and *A. mellifera*. The head and thorax of the three species were dissected at the 48th h of feeding. Total RNA was extracted from the head and thorax tissues after feed the three mixed oligosaccharides. The head and thorax of *A. camellia*, *A. cerana* or *A. mellifera* were dissected in 1.5 mL RNase centrifuge tubes as one sample (eight biological replicates were set for each honey bee species) and total RNA was extracted according to the instructions of the TransZol™ Up Plus RNA extraction kit (Beijing TansGen Biotechnology Co., Beijing, China). The final volume of complementary DNA (cDNA) prepared with a cDNA synthesis kit (Takara, Dalian, China) was 30 μL. Primers were designed by Primer 5.0 using the CDS sequences of *A. camellia* obtained from our lab sequence results, and the CDS sequences of *A. cerana* (ApisCC1.0) and *A. mellifera* (Amel_HAv3.1) genes downloaded from National Center for Biotechnology Information (NCBI). Sequences of primers for the GAL, GALK, GALT, and GALE genes of *A. camellia*, *A. cerana* and *A. mellifera* were listed in Table S8. All the primers were synthesized by Sangon Biotech (Shanghai, China). RT-qPCR amplification systems include 5 μL of SYBR® Premix ExTaqTM II (Takara Co. Beijing, China), 0.2 μL ROX (Takara Co. Beijing, China), 0.4 μL of forward primer (10 μM), 0.4 μL of reverse primer (10 μM), 3 μL nuclease-free water, and 1 μL of diluted template cDNA (400 ng/μL) in an RT-qPCR machine (ABI Q5, Fisher Scientific Inc., Waltham, MA, USA). Cycling conditions were 95°C for 45 s and 60°C for 1 min, followed by 40 cycles of 50°C heated to 90°C (1°C increase per 6 s). At the end of the reaction, according to the CT value of each target gene and the reference gene (GAPDH), the relative expression of the target gene was calculated by the $2^{-\Delta\Delta CT}$ method.[24]

Clustering relationship analysis of NAGA-like, GALK, GALT, and GALE genes in different honey bee genomes

Homologous Coding Sequence of *NAGA-like*, *GALK*, *GALT*, and *GALE* in the genomes of 15 bee species were selected for cluster analysis, with Drosophila melanogaster as the outgroup. Phylogenetic trees were constructed using the MEGA 7.0 software packages.

In vitro expression of four enzymes and *in vitro* digestion of three oligosaccharides and galactose

NAGA-like, GALK, GALT, and GALE were successfully expressed using the constructed plasmids with the coding regions of four genes into *E. coli* (*E. coli* BL21 (DE3)-PET-28A-(NAGA-like; GALK; GALT; GALE)). Finally, the conversion of galactose to UDP-glucose was simulated *in vitro* using these four enzymes. Synthesize *NAGA-like*, *GALK*, *GALT* or *GALE* genes respectively, prepare the following system according to Table S9 in a 0.2 mL EP tube, dilute the template 20-fold, and take 0.5 μL to amplify *NAGA-like*, *GALK*, *GALT* or *GALE*. Mix well, and then put it into the GeneAmp PCR System 2400 PCR equipment (Fisher Scientific Inc., Waltham, MA, USA) for amplification. Amplification conditions were 94°C for 1 min, followed by 30 cycles (98°C for 15 s, 58°C for 15 s and then 68°C 1 min). After storage at 68°C for 5 min, the final instrument temperature was reduced to 4°C to preserve the samples.

The PCR of *NAGA-like*, *GALK*, *GALT* or *GALE* products were recovered using the DNA Gel Recovery Kit (Dongsheng Biotech, Guangzhou, China), and the experimental procedures were referred to the reagent manufacturer's instructions. Photographs of PCR products during cut gel recovery are shown in Figure S1. Then, the PCR recovery products of *NAGA-like*, *GALK*, *GALT* or *GALE* and pET-28a+ plasmid (Fisher Scientific Inc., Waltham, MA, USA) was each taken in 15 μL, respectively, and were double digested with XhoI/BamHI (Takara, Dalian, China), and the enzymatic system was shown in Table S10. After mixing, the reaction was done at 37°C for about 3 h. Likewise, the enzymatic products of GAL, GALK, GALT or GAKLE were recovered using the DNA Gel Recovery Kit (Dongsheng Biotech, Guangzhou, China). Linking of the target fragment to the plasmid was performed using T4 DNA Ligase (Takara, Dalian, China), with the reagent addition system as described in Table S11, and sustained ligation at 16°C for 1 h.

5 μL of ligation product (GAL, GALK, GALT or GALE) was added to a 1 mL centrifuge tube with 50 μL of DH5α receptor cells in an ice bath. The tubes were then gently spun and mixed and incubated in an ice bath for 30 min. Heat shock was applied to the tubes in a water bath at 42°C for 90 s. Following this, the tubes were immediately transferred to an ice bath and incubated for 2 min. 200 μL of LB medium was added to each of the tubes, mixed, and incubated with shaking at 37°C for 1 h at 200 rpm. The liquid was spread uniformly on a Luria-Bertani (LB) medium containing kanamycin (100 μg/mL) in an ultra-clean bench at room temperature until the liquid was absorbed. Finally, the plates were inverted and transferred to a biochemical incubator at 37°C for overnight incubation (12 h).

Several single clones were picked from the plates and cultured in centrifuge tubes containing 3 mL of LB medium overnight (12 h) on a shaker to extract the plasmids, which were digested at 37°C for 2 h (the enzymatic reaction system as described in Table S12). The digested products (GAL, GALK, GALT, or GALE) were separated by electrophoresis on 1% agarose gel containing ethidium bromide (EB) and imaged by UVP gel imaging system (Figure S2).

The constructed expression plasmid (PET-28A-(GAL, GALK, GALT, GALE)) was transformed into E. coli BL21 (DE3) expressing bacteria, coated on LB plates containing the appropriate antibiotic (Kan, final concentration 50 μg/mL) and incubated in an inverted incubator at 37°C

overnight (12 h).Next, single colonies were picked from the plates and inoculated in 3 mL of LB liquid medium (containing Kan) in an ultra-clean bench and pre-incubated overnight (12 h) at 37°C on a 230 rpm shaker. In a 1 L triangular flask, 2 mL of pre-culture bacterial solution was added to 200 mL of LB medium (containing Kan) at a ratio of 1: 100 with a micropipette and incubated at 37°C on a shaking bed at 230 rpm for about 2-3 h, so that its OD_{600nm} reached 0.4-0.8.

Also in the ultra-clean bench, 1 mL of 100 mM inducer IPTG was added to the culture solution to give a final concentration of 0.5 mM. Incubation was induced overnight (12 h) at 18°C on a shaker at 180 rpm. After incubation, the triangular vials were removed from the shaker and placed on ice for 10 min, during which time they were shaken two or three times. Using a pipette, the bacterial solution was pipetted into a clean 50 mL centrifuge tube and centrifuged at 4 000 rpm for 10 min at 4°C. The supernatant was discarded. Repeat centrifugation until all organisms were collected.

Bacteria were resuspended in ice pre-cooled lysis buffer (50 mM NaH_2PO_4, 300 mM NaCl, 10 mM imidazole, adjusted to pH 7.4 with NaOH), trying to avoid air bubbles as much as possible, and 200 mL of bacterial solution was finally resuspended in 16 mL lysis buffer and shaken slowly for 1 h on ice. A total of 5 bottles were shaken, totaling 1 L, and finally resuspended in 80 mL lysis buffer, which was divided into two 50 mL centrifuge tubes, 40 mL in each tube, and then ultrasonicated on ice for 6 s, with 6 s intervals, 60 times, at 300 W. After ultrasonication, centrifugation was performed at 4° C for 30 min at 10,000 g. Transfer the supernatant to a clean centrifuge tube, add 1 mL of Ni-NTA beads (Kingsley Biotechnology, Shanghai, China) pretreated with lysis buffer, and shake slowly on ice for 1 h to fully bind the beads to the protein.

Mixed solution of beads-proteins was transferred to a Poly-Prep chromatography column (Bio-Rad, Hercules, CA, US) to allow beads to settle naturally. Washing with 8 mL wash buffer (50 mM NaH_2PO_4, 300 mM NaCl, 20 mM imidazole, adjusted to pH 7.4 with NaOH) was performed two times. Finally, the lower target proteins were eluted with elution buffer (50 mM NaH2PO4, 300 mM NaCl, 250 mM imidazole, adjusted to pH 7.4 with NaOH) into 1.5 mL EP tubes 3 times, 1 mL each time. Bradford assay was used to determine the protein concentration (Table S13) in each tube and then 12% SDS-PAGE was applied to detect the purification effect (Figure S3).

Analysis the efficiency of hydrolysis with three types of oligosaccharides with GAL *in vitro* by HPLC

Firstly, 100 mg each of mannotriose, raffinose and stacyose standards (Sigma, Missouri, US, LC-MS, > 99% pure) were dissolved in 5 mL of LC-MS-grade water, and then transferred to a 10 mL volumetric flask and fixed to scale line with LC-MS-grade water. Before testing, each standard solution was diluted according to the gradient shown in Table S14 and loaded into a 1.5-mL injection vial. The fitting degrees of the 3 saccharide standards were all $\geq$ 0.9992, indicating a strong linear relationship. The results are shown in Table S15.

The mother liquor containing two times the content of single oligosaccharide was prepared with reference to the content of manninotriose and raffinose and stachyose in *Camellia oleifera* nectar,[11] respectively, and then 1 mL of the mother liquor was added to each of the two 10 mL centrifuge tubes. One tube was used as a control with 1 mL of PBS, and the other tube was added with 1 mL of GAL solution (concentration with 0.8 mg/mL), and two tubes both to shake and mix. Three replicates were set up for each group. The oligosaccharide content in control and treatment groups were detected after 48 h (the digestion was stopped by heating the tubes at 80°C for 15 min to inactivate the enzyme) of digestion at room temperature using HPLC techniques (Agilent, Santa Clara, CA, USA). Samples were diluted 4-fold prior to testing.

Analyses were performed in a ZORBAX NH_2 column (4.6×250 mm, 5 μm, Agilent, USA). Mobile phase A: 67% acetonitrile in LC-MS-grade water (v/v); gradient elution program: 0.0-17.0 min, 100% A; flow rate: 1.0 mL/min; column temperature 35°C; post-run for 5 min. The amount injected into the liquid chromatography system was 10 μL.

To prepare mixed standards including galactose, galactose 1-phosphate, UDP-galactose and UDP-glucose standards (Sigma, Missouri, US, LC-MS, > 99% pure) were accurate weighed. Then, LC/MS-grade water was used to prepare mixed standard santonin and caffeine solutions of 0.16, 0.8, 4, 20 and 40 mg/mL, respectively, which were used to form the standard working curves of both (Table S16).

The masterbatch was prepared based on the total amount of galactose in *C. oleifera* nectar and the solvent was mass spectrometry grade water.[11] Take two 10 mL centrifuge tubes, add 1 mL of mother liquor to one tube and then add 3 mL PBS solution as control group. After adding 1 mL of mother solution to the other tube, 1 mL each of GALK, GALT and GALE solutions (GALK concentration with 1.5 mg/mL, GALt concentration with 0.9 mg/mL, GALE concentration with 1.0 mg/mL) were added sequentially as treatment groups. For each group of experimental sets, four biological replicates were established. The two groups of tubes were shaken and mixed and then digested until 48 h (the digestion was stopped by heating the tubes at 80°C for 15 min to inactivate the enzyme) and LC-MS (AB Sciex, 5600 Q-TOF/6500 Q-TRAP, Framingham, MA, USA) was applied to detect the concentration of galactose, galactose 1-phosphate, UDP-galactose and u UDP-glucose in all samples.

Separations were carried out using an ACQUITY UPLC® BEH C18 chromatographic column (2.1×100 mm, 1.7 μm, Waters, Milford, MA, USA). Analytes were separated by LC-MS-grade water (containing 25 mM CH_3COONH_4 and 25 mM NH_4OH) (A) and Acetonitrile (B) at a flow-rate of 0.3 mL/min. The linear gradient elution program was: 0.0∼1.0 min, 85% B; 1.1∼12.0 min, 65% B; 12.1∼15.0 min, 40% B; 15.1∼20.0 min, 85% B. The column was thermostated at 40°C and injection volume 5 μL.

Mass spectrometry conditions: electrospray ionization source (ESI). Ion-source temperature 600°C, ion-source voltage 5500 V, curtain gas 20 psi, nebulizer gas and auxiliary gas 60 psi. Scanning was performed using multiple reaction monitoring (MRM).

Data access

A. camellia genome at the chromosomal level have been deposited in NCBI under accession number PRJNA921724. All other data are included in the supplemental information.

QUANTIFICATION AND STATISTICAL ANALYSIS

Data analysis

All data are expressed as mean ± S.E.. For statistical analysis, one-way analysis of variance (ANOVA) was used to compare the relative gene expression level, enzyme activity and saccharide composition among the three honey bees followed by appropriate post hoc test was used to determine statistical significance ($P < 0.05$).