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Transcriptome and Taste Receptor Analysis of the Proboscis of *Apis mellifera* Collecting *Prunus persica* Pollen and *Taraxacum mongolicum* Pollen under Flowering-Overlap Competition

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Abstract: This study investigated the potential effects of *Taraxacum mongolicum*, a competing floral resource whose w flowering period overlaps with that of *Prunus persica*, on the foraging preferences of *Apis mellifera* for *Prunus persica* pollen, as well as the molecular mechanisms underlying gustatory perception in the proboscis. Proboscis samples were collected from worker bees that had collected *Prunus persica* pollen, *Taraxacum mongolicum* pollen, or no pollen. Transcriptome sequencing was performed on the Illumina NovaSeq 6000 platform to identify differentially expressed genes (DEGs) and to perform Gene Ontology (GO) functional annotation and Kyoto Encyclopedia of Genes and Genomes(KEGG) pathway classification. Gustatory receptor (GR) genes were identified through BLAST alignment, Open Reading Frame (ORF) prediction, and transmembrane domain analysis, after which a phylogenetic tree was constructed. The expression of GR genes were validated by quantitative real-time PCR (qRT-PCR). Compared with the control group (CH, no pollen), 616 DEGs were identified in the comparison between the dandelion (*Taraxacum mongolicum*) pollen-collecting group (GH) and CH (563 upregulated and 53 downregulated), 1,072 DEGs in the comparison between the peach (*Prunus persica*) pollen-collecting group (TH) and CH (973 upregulated and 99 downregulated), and 96 DEGs in the comparison between TH and GH (17 upregulated and 79 downregulated). GO functional annotation indicated that DEGs were primarily enriched in categories such as cellular processes, cellular anatomical entities, and binding. KEGG pathway analysis showed that DEGs were mainly involved in neuroactive ligand-receptor interaction, biosynthesis of unsaturated fatty acids, and fatty acid elongation. Five GR genes were identified in the proboscis transcriptome, all of which possessed complete open reading frames and seven transmembrane domains. Phylogenetic analysis showed that AmelGR10, AmelGR28b-like, AmelGR32a-like, and AmelGR68a-like clustered with *Drosophila* bitter taste receptors such as DmelGR33a and DmelGR66a, suggesting that they belong to the bitter taste receptor family. AmelGR64f clustered with sweet taste receptors such as DmelGR64a and DmelGR64e, suggesting that it belongs to the sweet taste receptor family. qRT-PCR validation showed that the expression patterns of the five GR genes were consistent with the RNA-seq results, confirming the reliability of the transcriptomic data. This study revealed the differential expression patterns of taste receptor genes in the proboscis of *Apis mellifera* when collecting *Prunus persica* pollen and *Taraxacum mongolicum* pollen. It provides a theoretical

basis for elucidating the trade-off between nutritional benefits and chemical defense in honeybees, for understanding the potential impact of overlapping floral resources such as *Taraxacum mongolicum* on *Prunus persica* pollination, and for optimizing flowering-period management and pollination in *Prunus persica* orchards.

Keywords: *Apis mellifera*; proboscis transcriptome; taste receptor genes; pollen collection; differential expression analysis

1. Introduction

Plants and pollinating insects have evolved mutualistic relationships through long-term coevolution [1]. Plants depend on pollinators for sexual reproduction, while pollinators acquire energy and nutrients from nectar and pollen [2]. In agricultural ecosystems, *Apis mellifera* is an important pollinator of spring-flowering fruit trees, such as *P. persica*. *P. persica* has a short flowering season, with a particularly brief peak-bloom period; successful pollination therefore depends heavily on high bee visitation frequency and strong flower constancy. Consequently, competition for pollinators during the flowering period may directly affect *P. persica* pollination [3, 4].

However, in *P. persica* orchards and the surrounding areas, other flowering plants often bloom concurrently with *P. persica*. Because these plants share pollinators with *P. persica*, they may reduce bee visitation to *P. persica* flowers and lower pollination efficiency by competing for the same pollinator pool.*Taraxacum mongolicum* is a common early-spring flowering plant in *P. persica* orchards; its spring flowering period overlaps with the peak bloom of *P. persica* [5], and its relatively long population-level flowering period allows it to provide pollen and nectar to bees both before and after the *P. persica* flowering period [6]. *T. mongolicum* may thus act as a competing floral resource, altering bee visitation frequency and foraging preference for *P. persica* flowers and potentially affecting *P. persica* pollination. Whether this ecological effect occurs,however, depends largely on bees' foraging decisions between *P. persica* and *T. mongolicum*.

Honeybee foraging preferences are shaped not only by the sugar rewards of nectar but also by the non-sugar components of pollen. Pollen, however, is not merely a "nutritional package": many plants accumulate secondary metabolites—including phenolamides, flavonoids, and terpenes—in their pollen. Although these compounds primarily defend plants against herbivorous insects, they may also influence pollinators' foraging decisions [7]. *T. mongolicum* pollen contains high levels of phenolamide compounds, among which N^1, N^5, N^{10}-tri-p-coumaroyl spermidine is a major component [8]. These compounds have been shown to exert antifeedant or growth-inhibitory effects in certain insects [9]. In contrast, relatively few studies have reported such defensive compounds in *P. persica* pollen, whereas its nutritional rewards appear more pronounced. Therefore, when choosing between foraging on *T. mongolicum* and *P. persica*,

honeybees may face a trade-off between obtaining nutritional rewards and avoiding chemically defended pollen. In commercial pollination and beekeeping, *Apis mellifera* has long been the dominant managed pollinator, and its foraging decisions rely heavily on the chemical sensory system. Gustatory receptors (GRs) constitute a key receptor family mediating contact chemoreception in insects; they are expressed in tissues that come into direct contact with substrates, such as the proboscis, tarsi, and antennae [10]. Unlike insects such as fruit flies, the honeybee GR family has not undergone significant expansion and remains small, comprising only about 10 annotated members [11]. Despite this, its members exhibit distinct functional specialization, enabling bees to discriminate among various monosaccharides, disaccharides, and some oligosaccharides, as well as to detect non-sugar components in pollen[12].Sweet receptors are responsible for assessing sugar rewards, whereas bitter receptors may underlie the detection of—and aversion to— plant secondary metabolites [13]. This functional division of labor allows honeybees to evaluate both the nutritional value of pollen and potential chemical risks before committing to ingestion[14].

The proboscis is the final chemical checkpoint prior to ingestion in honeybees, and its gustatory receptors are directly exposed to pollen and nectar [15]. GRs in the proboscis may therefore play a key role in enabling honeybees to distinguish *P. persica* pollen from *T. mongolicum* pollen and in shaping their foraging preferences.

From the perspective of proboscis gustatory perception, this study aimed to clarify how T. mongolicum—a competing floral resource with an overlapping flowering period—affects honeybee preference for collecting P. persica pollen, and thereby to provide a theoretical basis for elucidating the molecular mechanisms underlying floral-source selection in honeybees, as well as for optimizing floral resource management and pollination in P. persica orchards.

To date, however, direct molecular evidence is lacking as to whether the phenolamide compounds in *T. mongolicum* pollen influence honeybee foraging preferences for *P. persica* pollen by regulating GR expression in the proboscis, thereby potentially affecting *P. persica* pollination. Accordingly, this study used the proboscises of *Apis mellifera* workers that had foraged on *Prunus persica* pollen (TH), *T. mongolicum* pollen (GH), or no pollen (CH) as the experimental material. Transcriptome sequencing was performed on the Illumina NovaSeq 6000 platform to identify differentially expressed genes, followed by gustatory receptor (GR) gene identification and qRT-PCR validation. From the perspective of proboscis gustatory perception, this study aimed to clarify how *T. mongolicum*—a competing floral resource with an overlapping flowering period—affects honeybee preference for collecting *P. persica* pollen, and thereby to provide a theoretical basis for elucidating the molecular mechanisms underlying flower source selection in honeybees, as well as for optimizing floral resource management and pollination in *P. persica* orchards.

2 Materials and Methods

2.1.Test Insects

A. mellifera samples were collected from March 29 to April 8, 2026, in Liushang Village, Qiji Town, Yuncheng City, Shanxi Province, China (geographic coordinates: 35.0281°N, 110.5175°E). *P. persica* was the predominant tree species in the orchard, and early-spring herbaceous plant communities dominated by *T. mongolicum* occurred naturally within and around the orchard, covering approximately 0.2 ha. six colonies were housed in standard Langstroth hives and standardized to roughly the same strength (approximately 3,000–3,500 worker bees on four frames), each containing one queen and a small amount of of larvae, with virtually no pollen or nectar stores. When approximately 25% of the flowers in the *P. persica* orchard had bloomed, the colonies were transported to the site, and the six hives were arranged in a single row within the orchard, positioned between the *P. persica* trees and the *T. mongolicum* community. The following day, between 11:00 a.m. and 3:00 p.m., 200 worker bees were collected from each of three groups at the hive entrance: pollen foragers returning with *P. persica* pollen loads, pollen foragers returning with *T. mongolicum* pollen loads, and bees carrying no pollen. Immediately after collection, the proboscises of the bees were dissected on ice, rapidly frozen in liquid nitrogen, and stored at −80 °C for later use.

No plant specimens were collected in this study. The experimental material was the proboscis of *A. mellifera* workers. Foragers were grouped according to the pollen they were carrying or collecting. Specifically, foragers were assigned to the *P. persica* pollen-collecting group or the *T. mongolicum* pollen-collecting group based on the colour of the pollen loads carried in the corbiculae, which were visually matched to the pollen of the flowering plants present at the sampling site during the same period. Pollen-free foragers were assigned to the control group. No formal herbarium voucher specimen was prepared because no plant material was collected. Permission to access the sampling site and to handle honey bee colonies was obtained verbally from the landowner on the day of sampling (March 29–April 8, 2026) . No plant material was collected; therefore, no plant collection permit was required.

2.2 RNA Extraction, Library Preparation, and Transcriptome Sequencing

Total RNA was extracted from the proboscises of *A. mellifera* workers using the MJzol Animal RNA Isolation Kit (Majorivd) and purified with the RNAClean XP Kit (Beckman Coulter) and the RNase-Free DNase Set (QIAGEN). Samples were divided into three groups according to the type of pollen carried by the worker bees: *P. persica* pollen foragers (TH), *T. mongolicum* pollen foragers (GH), and pollen-free bees (CH). Each group consisted of three biological replicates (TH1–TH3, GH1–GH3, and CH1–CH3, respectively), with each replicate consisting of proboscises pooled from 200 worker bees. RNA integrity was assessed using an Agilent 2100 Bioanalyzer/Agilent 4200 TapeStation (Agilent Technologies), and RNA quantity and purity were measured using a Qubit 2.0 Fluorometer (Thermo Fisher Scientific) and a NanoDrop ND-2000

spectrophotometer (Thermo Fisher Scientific). After passing quality control, all samples were sent to Shanghai Bohao Biotechnology Co., Ltd. for transcriptome sequencing, and the RNA preparations returned after sequencing were subsequently used for qRT-PCR.

The mRNA sequencing library was constructed from purified total RNA following the manufacturer's protocol: mRNA isolation, fragmentation, first- and second-strand cDNA synthesis, end repair, 3' adenylation, adapter ligation, and fragment enrichment. Library construction was performed using the Dual-mode:mRNA:Library Prep Kit (YEASen, Cat. No. 12310ES96), and size selection was performed using HieffNGS DNA Selection Beads (YEASen, Cat. No. 12601ES56). Library concentration was measured using a Qubit 2.0 Fluorometer (Thermo Fisher Scientific), and fragment size distribution was assessed using an Agilent 4200 TapeStation (Agilent Technologies). After passing quality control, the libraries were sequenced on the Illumina NovaSeq 6000 platform with 150-bp paired-end reads (PE150).

2.3. Quality Control and Alignment of Transcriptome Sequencing Data

Clean reads from the nine transcriptome samples were aligned to the *A. mellifera* reference genome using HISAT2 software (default parameters). To functional characterize the differentially expressed genes (DEGs), Gene Ontology (GO) annotation was performed, and annotated genes were classified into three categories: biological process (BP), molecular function (MF), and cellular component (CC). In parallel, genes were assigned to KEGG pathways to identify differences in molecular functions and metabolic activities among the three pollen-collection groups (*P. persica* pollen, *T. mongolicum* pollen, and no pollen).,

2.4 Differential Expression Analysis

Gene expression profiles of the pollen-collection groups were compared with the no-pollen control (CH) to identify genes with significant expression changes. Read counts mapped to each gene were estimated using StringTie and used as the raw expression input for differential expression analysis with edgeR. Gene expression levels were normalized as FPKM (fragments per kilobase of transcript per million mapped reads) to ensure comparability across genes and samples. DEGs were identified using the following criteria: q-value ≤ 0.05 and $|\log_2 \text{FoldChange}| \geq 1$.

2.5 Screening and Analysis of Taste Receptor Genes

To identify gustatory receptor genes in the proboscis transcriptome, genes annotated as GRs were retrieved from the annotation results by keyword search. The sequences of these candidate GR genes were aligned against the NCBI non-redundant (nr) database using BLAST, and the top hits (species, gene name, and accession number) were recorded for confirmation. Open reading frames (ORFs) of the *A. mellifera* GR genes were predicted using NCBI ORF finder (https://www.ncbi.nlm.nih.gov/orffinder/), and the encoded amino acid sequences were submitted to TMHMM 2.0 (https://services.healthtech.dtu.dk/services/TMHMM-2.0/) to predict

transmembrane domains. A gene was ultimately confirmed as a GR if its encoded protein possessed seven transmembrane domains and a conserved motif in the C-terminal region.

2.6. Construction of a Phylogenetic Tree of Gustatory Receptor Genes

To investigate the evolutionary relationships of the five *A. mellifera* AmelGRs with GRs from other insects, we constructed phylogenetic trees using amino acid sequences of GRs from Hymenoptera and *Drosophila melanogaster*. First, amino acid sequences of GRs from six Hymenopteran species (*Apis cerana cerana*, *Apis dorsata*, *Apis laboriosa*, *Apis florea*, *Osmia lignaria*, *Osmia bicornis bicornis*), as well as from the bumblebee *Bombus terrestris*, were retrieved from the NCBI database. Multiple sequence alignment was performed in MEGA X, and a phylogenetic tree was constructed using the neighbor-joining (NJ) method in MEGA 7.0. Based on the resulting phylogeny, the *A. mellifera* GRs were named according to their orthologous relationships with known hymenopteran GRs. Next, a second tree was constructed from the five AmelGRs together with 52 GRs of *D. melanogaster* to infer their functional relationships by homology with functionally characterized fly GRs.

2.7 Real-Time Quantitative Fluorescence Analysis

To validate the reliability of the RNA-seq data, five GR genes (AmelGR10, LOC100576167, LOC413684, LOC102656321, and LOC100577091) were selected for qRT-PCR validation. The *A. mellifera* Arp1 gene (GenBank accession number: NM_001185145.1) served as the internal reference.. Total RNA was reverse-transcribed into cDNA, which was then used as the template for qPCR with TB Green® Premix Ex Taq™ (Tli RNaseH Plus) following the manufacturer's instructions. Each 10-μL reaction contained 5 μL of TB Green Premix Ex Taq, 0.5 μL each of forward and reverse primers (10 μmol/L), 500 ng of cDNA template, and nuclease-free water. The thermal cycling program was 95 °C for 3 min, followed by 40 cycles of 95 °C for 5 s, 57 °C for 30 s, and 72 °C for 30 s, with a subsequent melting curve analysis (95 °C for 10 s, 52 °C for 5 s). Each group included three biological replicates, each with three technical replicates.Relative expression levels were calculated using the $2^{(-\Delta\Delta CT)}$ method and are presented as $\log_2$ fold change. Primer information is provided in Table 1.

Table 1 Primer Sequences

Genes	Forward primer sequences	Reverse primer sequences
Arp1	TGCTGCACTCGTAGTTGACAATGG	ACCCTGGTGGCGTGGTCTTC
Gr10	GAAGCAGCGGCTTACTTTCAA	GAATATGCTCACCGAAAGCAGG
LOC100576167 (GR32a-like)	ACCACGCCAGCGCTTATTA	TTTCAAGCACGTGACCCAGA
LOC413684 (GR64f)	ACGTGCGCTGTTACCTTACT	CAGCCACCGCAAGCATAAAA
LOC102656321 (GR28b-like)	CACGGGAACTTAACCAAGCATATAA	TGTGTTGTTACTTCTACCTCGTGA

LOC100577091（GR68a-like）	TGTGACGGACGTAAGTAAAGAGA	AAGGTAGTAATTTTGCACGTCATCT

3 Results and Analysis

3.1. Transcriptome of the *A. mellifera* Worker Bee Proboscis

To analyze the gene expression profiles in the proboscises of workers foraging on different floral resources, transcriptome sequencing was performed on the Illumina NovaSeq 6000 platform. Nine cDNA libraries were constructed:three from P. persica pollen foragers (TH1–TH3), three from T. mongolicum pollen foragers (GH1–GH3), and three from pollen-free bees (CH1–CH3). Sequencing of the nine libraries yielded 398,337,014 raw reads in total. After quality filtering, 374,366,603 clean reads were retained, with Q20 values of no less than 94.36% and G+C contents ranging from 35% to 39% (Table 2), indicating that the sequencing data were of high quality and suitable for subsequent bioinformatics analysis.

Table 2 Statistics and evaluation of proboscis transcriptome data of *Apis mellifera ligustica* workers

Sample	Raw Data Read	Valid Data Read	Valid Ratio(reads)	Q20%	G+C (%)
CH1	52797258	48934272	92.68	95.72	36
CH2	48984436	44882531	91.63	95.34	36
CH3	42639428	38816702	91.03	94.99	35
GH1	38675440	37394876	96.69	97.18	39
GH2	46205694	44643853	96.62	97.3	38
GH3	35880516	34530965	96.24	97.1	38
TH1	49596418	47692036	96.16	97.32	38
TH2	38834420	37135052	95.62	97.11	39
TH3	44723404	40336316	90.19	94.36	37

GH1–GH3: proboscis samples from *A. mellifera* workers that had collected *T. mongolicum* pollen, with three biological replicates; TH1–TH3: proboscis samples from *A. mellifera* workers that had collected *P. persica* pollen, with three biological replicates; CH1–CH3:samples from *A. mellifera* workers that had collect no pollen, with three biological replicates. The same applies below.

3.2. Alignment of proboscis transcriptome reads to the *A. mellifera* reference genome

Clean reads from each sample were aligned to the *A. mellifera* reference genome (Amel_HAv3.1.62) using HISAT2. As shown in Table 3, the mapping rates of the nine libraries ranged from 72.50% to 89.51%. The CH group showed the highest mapping rate (89.21%–89.51%), followed by the *T. mongolicum* pollen group (GH; 78.05%–79.39%), while the P. persica pollen group (TH) had the lowest (72.50%–77.75%). Mapping rates above 70% are generally

considered acceptable for RNA-seq data. Moreover, for all samples, more than 79% of the mapped reads fell within exonic regions, and the proportion of intronic reads exceeded that of intergenic reads (7.72%–10.08%). These results indicate that the reference genome annotation used in this study is of high quality and that the sequencing data are reliable, meeting the requirements for subsequent gene expression quantification and differential expression analysis.

Table 3 Statistical results of the proboscis transcriptome sequence alignment with the reference genome of *A. mellifera* workers

Samples	Number of total reads	Mapped reads（%）	Unique Mapped reads（%）	exon	intron	intergenic
CH1	45901696	89.51	88.35	82.58	9.70	7.72
CH2	41527762	89.4	88.19	82.12	9.72	8.16
CH3	36619880	89.21	87.90	82.00	9.77	8.23
GH1	35414938	78.05	77.39	80.82	10.18	9.00
GH2	42619668	79.39	78.72	80.56	10.25	9.19
GH3	32693318	78.88	78.22	80.48	10.31	9.21
TH1	45282786	76.70	76.14	79.25	10.78	9.97
TH2	35079744	72.50	71.91	79.10	10.82	10.08
TH3	37703350	77.75	77.30	79.86	10.55	9.59

3.3.Identification of differentially expressed genes

Hierarchical clustering of DEGs (Figure 1) revealed distinct gene expression profiles among the three groups, whereas biological replicates within each group clustered closely together, confirming high reproducibility of the data. Differential expression analysis was then performed with edgeR, using CH as the reference. Compared with CH, 616 DEGs were identified in GH, including 563 upregulated and 53 downregulated genes; 1,072 DEGs were identified in TH, including 973 upregulated and 99 downregulated genes. In the TH vs. GH comparison, 96 DEGs were detected, of which 17 were upregulated and 79 were downregulated in TH relative to GH (Figure 2).

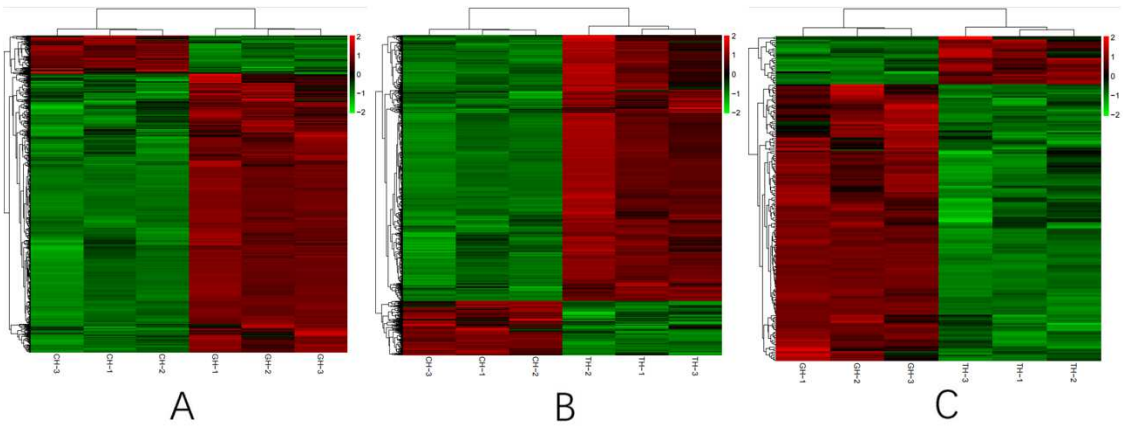


Fig. 1. Hierarchical clustering heatmap of differentially expressed genes in the proboscises of *A. mellifera* workers from the three pollen-collection groups (TH, GH, and CH).

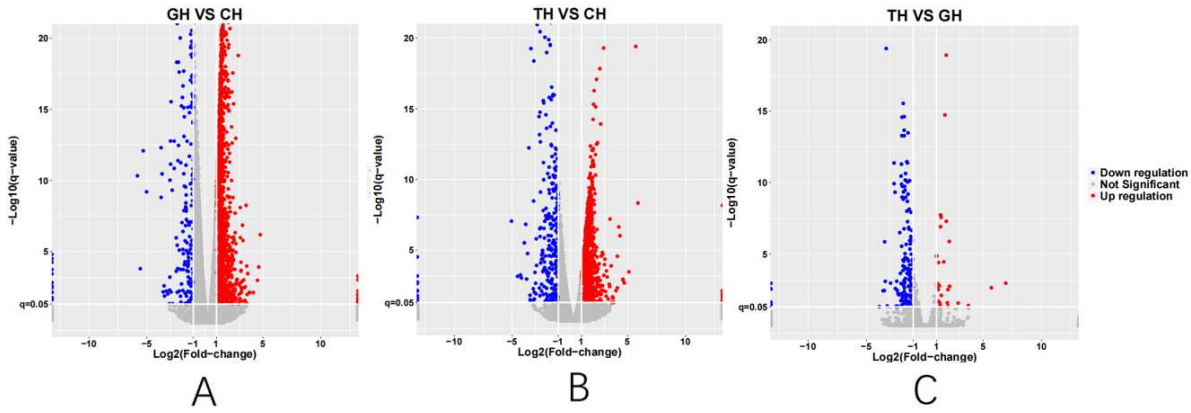


Fig. 2. Volcano plots of differentially expressed genes in the proboscises of *A. mellifera* workers from the three pollen-collection groups.

Note:(A) GH vs. CH; (B) TH vs. CH; (C) TH vs. GH. The horizontal dashed line indicates the significance threshold (q-value = 0.05), and the vertical dashed lines indicate the fold-change threshold ($|\log_2 \text{FoldChange}| = 1$).

3.4. Functional annotation of DEGs in the proboscis transcriptome of *A. mellifera* pollen-collecting Workers

To explore the biological processes and metabolic pathways associated with pollen foraging, GO annotation and KEGG pathway analysis were performed on the DEGs identified in the GH and TH groups. In total, the DEGs were assigned to 31 GO functional categories across the three main ontologies: biological process (BP), molecular function (MF), and cellular component (CC). Among these, the most highly represented categories were cellular process (BP), cellular anatomical entity (CC), and binding (MF) (Figure 3).

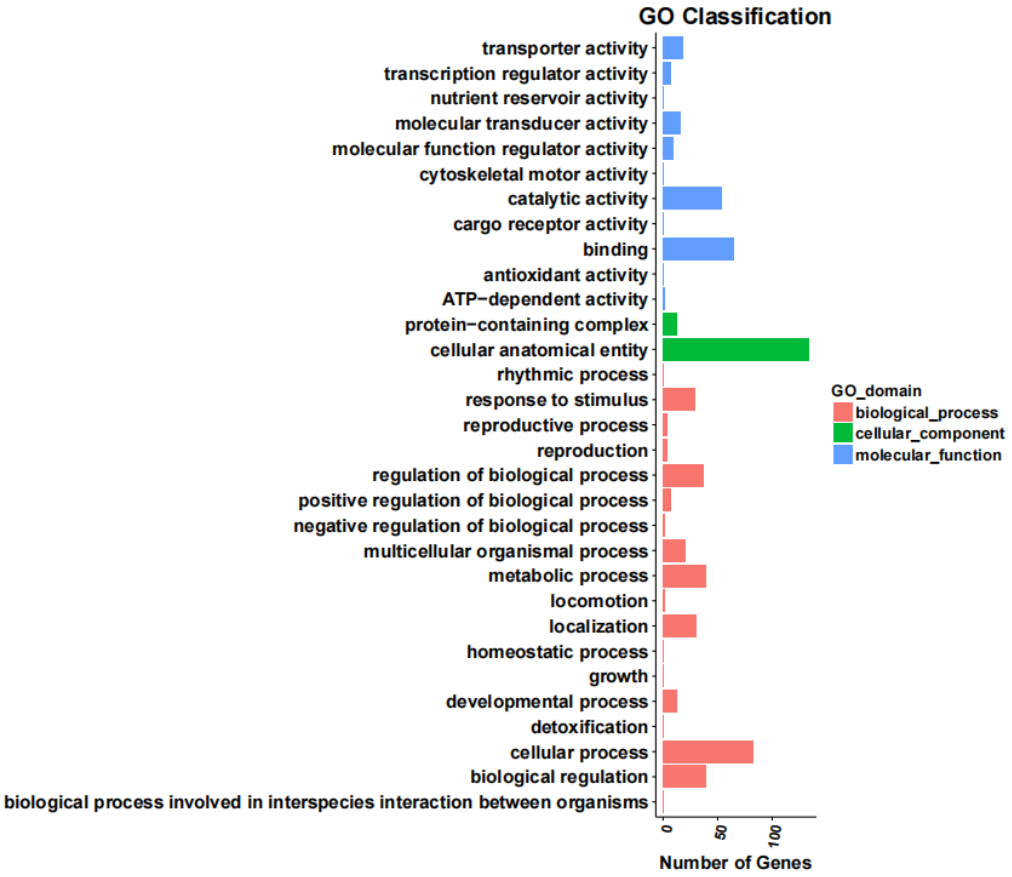


Fig. 3 GO functional annotation of differentially expressed genes between the proboscis transcriptomes of *A.mellifera* workers collected *T. mongolicum* pollen and those that *P. persica* pollen.

A comparison with the KEGG database showed that five genes were involved in the neuroactive ligand-receptor interaction pathway, fourin the biosynthesis of unsaturated fatty acids pathway, fourin the fatty acid elongation pathway, and two in the purine metabolism pathway (Figure 4).

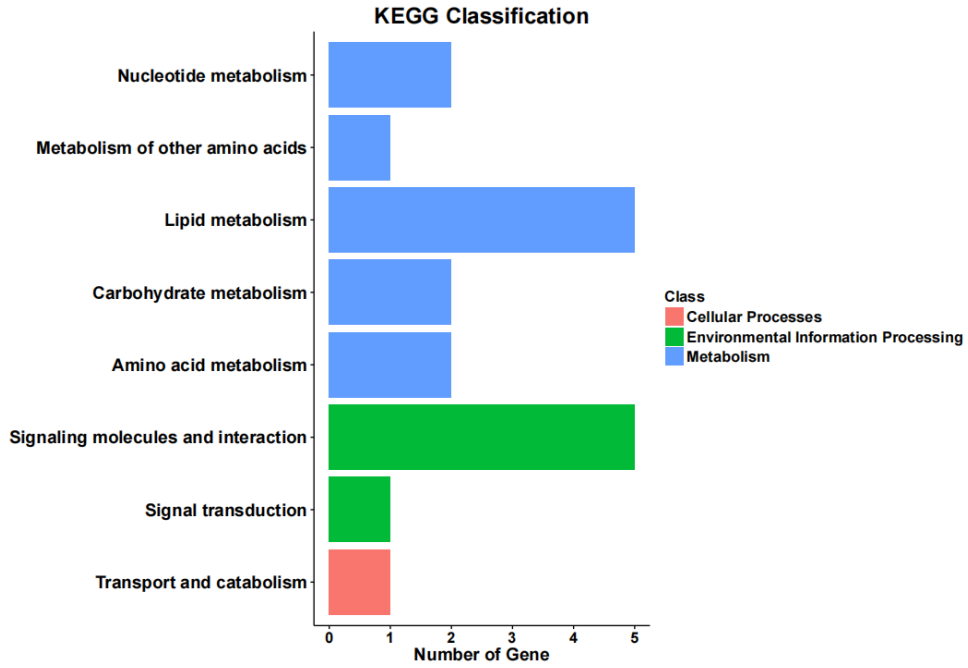


Fig. 4 KEGG functional annotation of differentially expressed genes between the proboscis transcriptomes of *A. mellifera* workers collected *T. mongolicum* pollen and those that *P. persica* pollen.

3.5. Sequences of Candidate Gustatory Receptor Genes in *A. mellifera* Worker Bees

Analysis of the *A. mellifera* proboscis transcriptome identified a total of five candidate genes annotated as gustatory receptors. BLAST alignment, open reading frame (ORF) prediction, and transmembrane domain analysis showed that all five genes possessed complete ORFs. Among them, AmelGR10 (LOC725297) had the longest ORF of 1,086 bp and exhibited 99.82% sequence identity with the *A. mellifera* gustatory receptor protein 10 (accession number: NM_001242994.1); LOC100576167 had the longest ORF of 1,233 bp, showing 100% sequence identity with the *A. mellifera* gustatory and pheromone receptor 32a-like (accession number: XM_016914041.2); LOC413684 had the longest ORF of 1,413 bp, with 100% sequence identity to the *A. mellifera* sweet gustatory receptor 64f (accession number: XM_397125.7); LOC102656321 had the longest ORF of 498 bp, with 100% sequence identity to the uncharacterized protein LOC102656321 from *A. mellifera* (accession number: XM_026445991.1); LOC100577091 had the longest ORF of 1,287 bp and showed 100% sequence identity with the uncharacterized protein LOC100577091 (accession number: XM_026444604.1) from *A. mellifera*. Based on these results, the five genes were identified as gustatory receptor genes.

3.6. Construction of a Phylogenetic Tree of AmelGR Gustatory Receptors

A phylogenetic tree was constructed based on amino acid sequences using the neighbor-joining (NJ) method, as shown in Figure 5. The *A. mellifera* gustatory receptor AmelGR10 is homologous to AcerGR10 from the *A. c. cerana* and AfloGR10 from the *Apis florea*, and they cluster closely within the same subclade. AmelGR32a-like clusters with AcerGR32a-like from the *A. c. cerana*, AdorGR32a-like from the *Apis dorsata*, AlabGR32a-like from the *Apis*

laboriosa, and AfloGR32a-like from the *Apis florea* on the same branch, indicating that this gene is highly conserved within the genus Apis. AmelGR64f clusters with AcerGR64f, AdorGR64f, AlabGR64f, and AfloGR64f on the same branch. AmelGR28b-like clusters with AcerGR28b-like, AdorGR28b-like, and AlabGR28b-like, and is homologous to BomGR28b from *Bombus*, further confirming its membership in the GR28b subfamily. AmelGR68a-like clusters with AcerGR68a-like, AlabGR68a-like, and AfloGR68a-like, exhibiting a high degree of sequence homology with the GR68a family. These results indicate that the five *A. mellifera* gustatory receptor genes identified in this study are highly conserved among Hymenoptera and all belong to known gustatory receptor subfamilies.

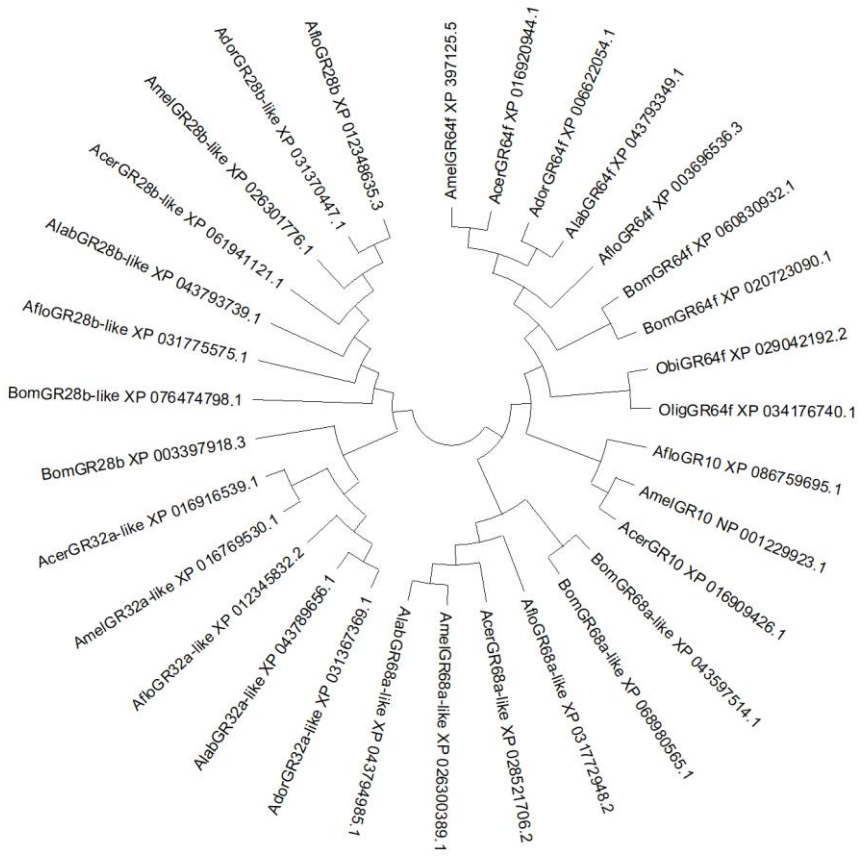


Fig. 5 Phylogenetic tree of AmelGRs from *A. mellifera* ligustica and GRs from other hymenopteran insects based on amino acid sequence constructed using neighbor-joining method

Protein origin species: Amel: *A．m． ligustica*; Acer: *A． cerana cerana*; Ador *A．*dorsata; Alab: *A．laboriosa;* Aflo: *A． florea*; Olig: *Osmia lignaria*; Obic: *Osmia bicornis bicornis；* Bom *Bumblebee, bombus*

A phylogenetic tree was constructed by combining the amino acid sequences of 52 gustatoryreceptor proteins from the fruit fly *D. melanogaster* with those of the five *A. mellifera* gustatory receptor proteins identified in this study, as shown in Figure 6. AmelGR10 clusters with DmelGR28a and DmelGR28b on the same branch; AmelGR28b-like is homologous to DmelGR8a and DmelGR98a and clusters closely with them; AmelGR32a-like and AmelGR68a-like cluster with the bitter taste receptors DmelGR33a and DmelGR66a on the same branch; AmelGR64f clusters with the sweet gustatory receptors DmelGR64a, DmelGR64e, and DmelGR64f on the

same branch. Based on the BLAST alignment results and the clustering relationships of these AmelGRs with known functional receptors in *D. melanogaster* , it is speculated that AmelGR10, AmelGR28b-like, AmelGR32a-like, and AmelGR68a-like may belong to the bitter gustatory receptor family, while AmelGR64f may belong to the sweet gustatory receptor family.

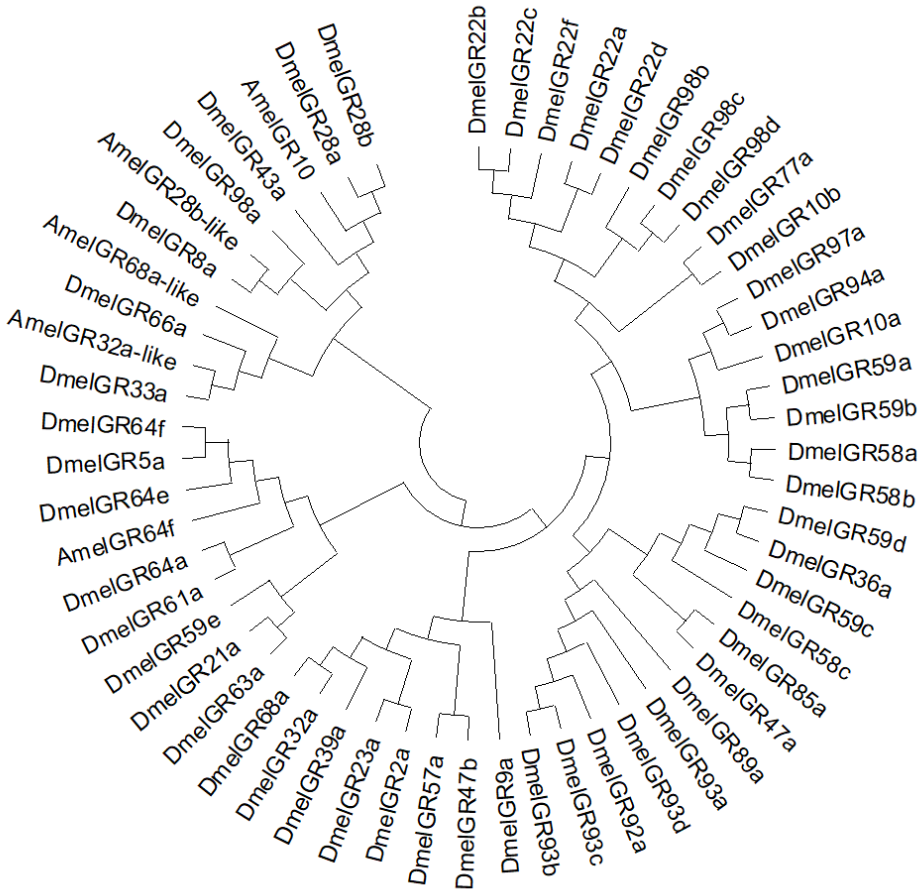


Fig. 6 Phylogenetic tree of Amel GRs from *A. mellifera* and GRs from *D. melanogaster* based on amino acid sequence constructed using neighbor-joining method

3.7. Validation of Candidate Gustatory Receptor Genes by qRT-PCR

To further validate the accuracy and reliability of the transcriptomic data, the expression levels of the gustatory receptor genes identified in the transcriptomic analysis were verified by qRT-PCR. As shown in Figure 7, the expression patterns obtained by qRT-PCR were highly consistent with the corresponding FPKM values from RNA-seq, confirming that the transcriptome sequencing data are accurate and reliable (Figure 7).

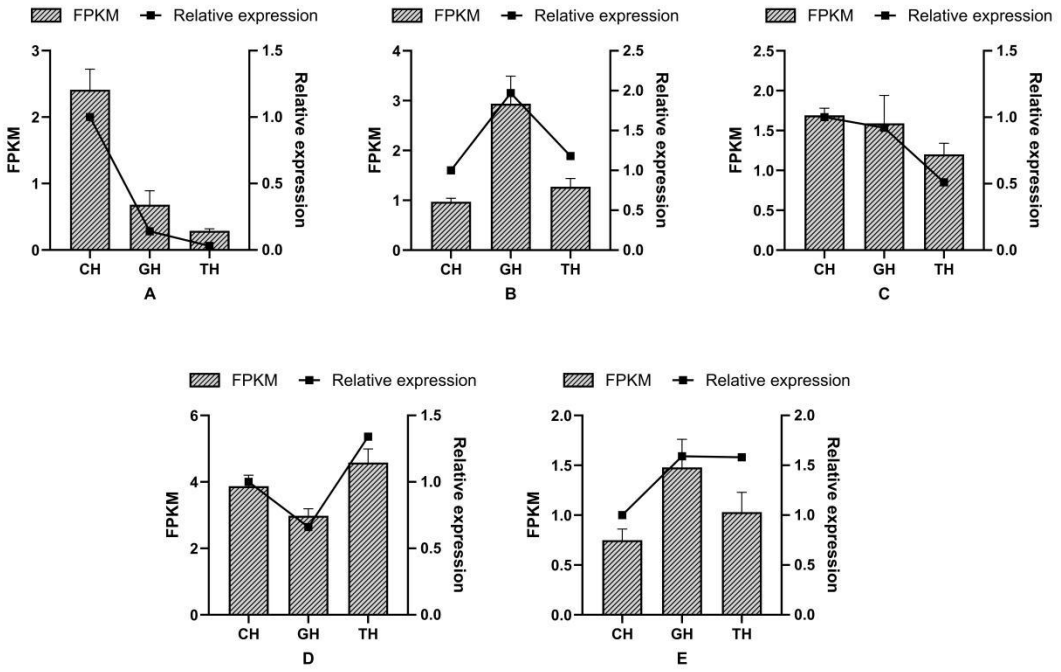


Fig. 7 Validation of candidate gustatory receptor genes by qRT-PCR. Relative expression levels of five candidate gustatory receptor genes — (A) Gr10, (B) LOC100576167, (C) LOC413684, (D) LOC102656321, and (E) LOC100577091 — in bees collecting different pollen types. Different letters above bars indicate significant differences ($P < 0.05$). Data are presented as mean ± SE.

4 Discussion

In this study, we identified five gustatory receptor genes from the proboscis transcriptome of A. mellifera workers and compared their expression patterns among workers that collected *P. persica* pollen, those that collected *T. mongolicum* pollen, and those that did not collect pollen. The number of differentially expressed genes (DEGs), the number between the *P. persica* pollen-collecting group and the control group (1,072) was markedly higher than that between the *T. mongolicum* pollen-collecting group and the control group (616), whereas only 96 DEGs were detected in the direct comparison between the two pollen-collecting groups. The larger number of DEGs in theP. p*ersica* group suggests that this pollen induces more extensive perturbation of the *A. mellifera* proboscis transcriptome, which may be related to its carbohydrate, amino acid, or lipid composition. In contrast, the relatively few DEGs in the direct comparison between the two pollen-collecting groups suggests that pollen-specific responses are concentrated in a small number of key receptors and metabolic pathways. Notably, this direct comparison revealed far more downregulated than upregulated genes, indicating that, relative to workers collecting *P. persica* pollen, those collecting *T. mongolicum* pollen exhibit greater gene downregulation in the proboscis. This may be associated with the inhibitory response triggered by phenolamide defense compounds in *T. mongolicum* pollen [16]. KEGG pathway analysis revealed that the DEGs were primarily enriched in neuroactive ligand-receptor interaction, biosynthesis of unsaturated fatty

acids, fatty acid elongation, and purine metabolism. The neuroactive ligand-receptor interaction pathway is directly related to taste signal recognition and transmission, indicating that the proboscis is regulated at the level of neural signal transduction upon contact with different pollen types [17]. The enrichment of fatty acid metabolism pathways may reflect the perception of and metabolic adaptation to lipids in *T. mongolicum* pollen [18, 19]. Compared with the immune and energy metabolism pathways commonly enriched in the antennal transcriptomes, the DEGs identified in the proboscis in this study were concentrated mainly in neuroactive ligand-receptor interaction and lipid metabolism. Previous studies on the proboscis of bees collecting pear and rapeseed pollen have reported pronounced enrichment of the oxidative phosphorylation pathway; however, this pathway was not play a dominant in our analysis. This discrepancy may reflect differences in pollen combinations, experimental design, and analytical parameters, and further suggests that the metabolic emphasis of proboscis responses varies with the pollen source being collected [20].

Phylogenetic analysis indicated that, among the five identified gustatory receptor genes, *AmelGR64f* clustered on the same branch as the *Drosophila* sweet gustatory receptors *DmelGR64a*, *DmelGR64e*, and *DmelGR64f* , suggesting that it belongs to the sweet gustatory receptor family. In contrast, *AmelGR10*, *AmelGR28b-like*, *AmelGR32a-like*, and *AmelGR68a-like* cluster with the *Drosophila* bitter gustatoryreceptors *DmelGR33a*, *DmelGR66a*, and related bitter gustatory receptors, indicating that they belong to the bitter gustatory receptor family. The expression trends of the five GR genes measured by qRT-PCR were consistent with the RNA-seq quantification results, confirming the reproducibility of the transcriptomic expression profiles. Notably, the candidate bitter gustatory receptor genes were not uniformly upregulated in the *Taraxacum mongolicum* pollen-collecting group: *AmelGR10* and *AmelGR28b-like* were downregulated, whereas AmelGR32a-like and AmelGR68a-like were upregulated. This differentiatial response suggests that the bitter gustatory receptors in the honeybee proboscis do not respond uniformly to plant defense compounds, and that different subtypes may serve distinct recognition functions [21]. Functional studies in *Drosophila* have shown that GR33a/GR66a can recognize various bitter ligands, while GR28b mediates the avoidance response to saponins [22]. Based on these findings, we hypothesized that *AmelGR32a-like* and *AmelGR68a-like* may be involved in recognizing phenolamide compounds in *T. mongolicum* pollen and triggering an avoidance response, whereas the downregulation of *AmelGR10* and *AmelGR28b-like* may reflect adaptive regulation or receptor desensitization following prolonged exposure, thereby preventing the taste system from overreacting to a single defensive signal [23].

The candidate sweet gustatory receptor gene *AmelGR64f* was downregulated in both the *T. mongolicum* and *P. persica* pollen-collecting groups compared with the control group, with stronger downregulation in the *P. persica* pollen-collecting group. These results suggest that sweet gustatory receptors expression in the proboscis does not simply reflect the nutritional value of pollen, but may also be modulated by pollen the carbohydrate composition, feeding duration, and

receptor desensitization [24, 25]. The reduced expression of *AmelGR64f* in bees feeding continuously on *P. persica* pollen may represent feedback regulation that prevents excessive activation of the taste system [26, 27], whereas its downregulation in bees feeding on *T. mongolicum* pollen may be associated with receptor desensitization following exposure to phenolamide defense compounds rather than with pollen palatability perse [28]. These findings are consistent with the nutrition–defense trade-off framework proposed in the Introduction: the foraging choice between *P. persica* and *T. mongolicum* pollen is not determined solely by nutritional content but reflects an integrated assessment mediated by both sweet and bitter gustatory receptors [29].

From an ecological perspective, *T. mongolicum* is a common early-spring flowering plants found in and around *P. persica* orchards. Its spring flowering period overlaps with the peak bloom of *P. persica* and is relatively long, enabling it to provide pollen and nectar to honeybees both before and after *P. persica* flowering. *T. mongolicum* may therefore compete with *P. persica* as a floral resource, influencing honeybee visitation frequency and foraging preference for *P. persica* flowers and potentially affecting its pollination. Previous studies have shown that phenolamide compounds in *T. mongolicum* pollen (such as N^1,N^5,N^{10}-tri-p-coumaroyl spermidine) exert significant antifeedant effect on generalist bumblebees, reducing both pollen collection and offspring production [30]. In this study, some candidate bitter gustatory receptor genes were upregulated in the *T. mongolicum* pollen-collecting group, suggesting that honeybees enhance their ability to recognize this class of defensive compounds [31]. Nevertheless, honeybees still successfully collected T. mongolicum pollen, indicating that, in early-spring *Prunus persica* orchards, they may not completely avoided *T. mongolicum* but instead made a trade-off between defensive signals and nutritional needs [32]. This "recognition without complete avoidance" phenomenon reflects a dynamic balance between chemical defense and nutritional rewards and further indicates that the honeybee taste system is plastic, adjusting foraging decisions according to environmental conditions and nutritional status [33, 34].

In this study, the type of pollen collected influenced *AmelGR64f* expression in the proboscis, whereas previous antennal studies have more commonly associate this gene with nectar sugar recognition. This suggests that the same GR gene may serve different functional roles across tissues: the antennae specialize in detecting long-distance chemical cues, whereas the proboscis is more directly involved in the final evaluation of feeding decisions. Furthermore, the known sweet gustatory receptors *AmelGR43a* was not detected in the proboscis transcriptome in this study. Although AmelGR43a has been reported to mediate fructose recognition [35], its expression in the proboscis remains unclear; its absence here may reflect tissue-specific differences in GR expression profiles (e.g., between proboscis and antennae) or the detection sensitivity of transcriptome sequencing.Overall, this study reveals the differential responses of GR genes in the proboscis of *A.mellifera* to *P. persica* versus *T. mongolicum* pollen, providing new molecular evidence for the trade-off between nutritional benefits and chemical defense in honeybees. From

an applied perspective, competing floral resources such as *T. mongolicum*, whose flowering period overlaps with that of *P. persica* trees, may indirectly affect *P. persica* pollination by altering bees' foraging preferences. Orchard management during the flowering period should therefore take into accountthe potential influence of *T. mongolicum* and other co-flowering weeds to optimize *P. persica* pollination.

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Ethics approval and consent to participate: Ethical review and approval were waived for this study because it involved the use of invertebrate animals (honey bees, *Apis mellifera*). This decision is in accordance with the official policy of our institution's Animal Care and Use Committee, as well as international guidelines (e.g., the International Committee of the Journal of Apicultural Research guidelines), which do not mandate ethical approval for research on insects. Although formal approval was not required, all research was conducted with a strong commitment to the principles of replacement, reduction, and refinement (the 3Rs) where applicable. We minimized disturbance to the colonies, used non-invasive observational and sampling methods where possible, and ensured that any necessary handling was performed carefully and swiftly to avoid undue stress or harm to the bees. The study did not involve any endangered or protected species. Permission to access the sampling site and to handle honey bee colonies was obtained verbally from the landowner on the day of sampling (March 29–April 8, 2026). No plant material was collected; therefore, no plant collection permit was required.

Informed Consent Statement: Not applicable. This study did not involve human subjects.

Availability of data and materials: The data presented in this study are openly available in NCBI Sequence Read Archive (SRA) at https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1543533, BioProject accession PRJNA1543533 .

Consent for publication: Not applicable.

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