Chemosensory genes in the antennal transcriptome of two syrphid species, *Episyrphus balteatus* and *Eupeodes corollae* (Diptera: Syrphidae)

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**Abstract**

**Background:** Predatory syrphid larvae are an important natural enemy of aphids in cotton agro-ecosystems in China. Their behaviors in prey foraging, localization and oviposition greatly rely on the perception of chemical cues. As a first step to better understand syrphid olfaction at the molecular level, we have performed a systematic identification of their major chemosensory genes.

**Results:** Male and female antennal transcriptomes of *Episyrphus balteatus* and *Eupeodes corollae* were sequenced and assembled using Illumina HiSeq2000 technology. A total of 154 chemosensory genes in *E. balteatus* transcriptome, including candidate 51 odorant receptors (ORs), 32 ionotropic receptors (IRs), 14 gustatory receptors (GRs), 49 odorant-binding proteins (OBPs), 6 chemosensory proteins (CSPs) and 2 sensory neuron membrane proteins (SNMPs) were identified. In *E. corollae* transcriptome, we identified 134 genes including 42 ORs, 23 IRs, 16 GRs, 44 OBPs, 7 CSPs and 2 SNMPs. We have provided full-length sequences of the highly conserved co-receptor Orco, IR8a/25a family and carbon dioxide gustatory receptor in both syrphid species. The expression of candidate OR genes in the two syrphid species was evaluated by semi-quantitative reverse transcription PCR. There were no significant differences of transcript abundances in the respective male and female antenna, which is consistent with differentially expressed genes (DEGs) analysis using the FPKM value. The sequences of candidate chemosensory genes were confirmed and phylogenetic analysis was performed.

**Conclusions:** This research comprehensively analyzed and identified many novel candidate chemosensory genes regarding syrphid olfaction. It provides an opportunity for understanding how syrphid insects use chemical cues to conduct their behaviors among tritrophic interactions of plants, herbivorous insects, and natural enemies in agricultural ecosystems.

**Keywords:** *Episyrphus balteatus*, *Eupeodes corollae*, Transcriptome, Chemosensory genes, Odorant receptors, Syrphid olfaction

**Background**

Natural enemies are important in controlling the population of insect pests in agricultural ecosystems [1–6]. The presence of natural enemies is detected through chemical cues emitted by prey and host plants, alone or in association, and received by the antennal sensory system in the multi-trophic environment to result in various behavioral choices including prey forage, localization, oviposition and escape [7–17]. Recently the study of insect peripheral sensory system and chemosignal transduction has experienced considerable progress due to the development of bioinformatics-based approaches and protein function prediction methods [18]. In particular, a large amount of information is provided by antennal transcriptome projects from various insect taxa [19–28].

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In general, the process of chemoreception, including olfaction and taste, involves several families of genes, including odorant receptors (ORs), ionotopic receptors (IRs), and gustatory receptors (GRs) [20, 29–31]. In addition, odorant binding proteins (OBPs), chemosensory proteins (CSPs) and sensory neuron membrane proteins (SNMPs) also play crucial roles in chemoreception [32–36]. The insect chemoreceptor superfamily including ORs and GRs was first identified in the Drosophila melanogaster genome [18, 21]. Insect odorant and gustatory receptors were once thought to be G-protein-coupled receptors just like ORs in worms and vertebrates, but subsequent studies have shown a lack of homology to vertebrate ORs [37]. One such superfamily encoding ORs is highly divergent across insect taxa with sequences and frequencies varying to a large extent [18, 38, 39]. ORs are broadly tuned to alcohols, ketones, and esters generally present in the environment [40, 41]. Another family encoding GRs, or receptors for taste or contact stimuli, is also very divergent across insect taxa [31]. On the contrary, one example of exceptionally conserved GRs are GR21a and GR63a, which work together as a CO₂ receptor in Drosophila [42]. Such chemoreceptors play an important role in host seeking behaviors in many insects but, especially seen in mosquitoes [43, 44]. A new insect chemosensory family was identified recently and given the name ionotropic receptors (IRs). These IRs belong to the ionotropic glutamate receptor superfamily (iGLuRs) and were identified in both the olfactory and gustatory systems [30, 45]. IRs are more greatly conserved than ORs and GRs but considerable variations can be observed in ligand-binding domains. They are mainly tuned to acids, amines and other odorants that are not detected by ORs [30, 45, 46].

Since chemosensory gene families were characterized in two important model species, D. melanogaster and Anopheles gambiae [47, 48], a growing number of chemosensory genes have been identified from many Dipteran species, such as Musca domestica [49], Bactrocera dorsalis (genome: assembly ASM78921v2), Calliphora stygia [50], Glossina morsitans morsitans [51], and Mayetiola destructor Say [20]. Protein prediction methods have been the first step for functional identification of chemosensory genes. All information regarding insect chemosensory was obtained bioinformatically and has been beneficial in understanding insect processing of diverse volatile compounds and cross-species differences in chemical communication.

The syrphids belong within the Diptera order and their larvae are aphid-specific natural enemies [1, 2, 4, 5, 8]. Due to the larvae’s agricultural importance via potential applications, several reports have been published on the chemical ecology of these insects. In Episyrphus balteatus DeGeer, larvae may use a sesquiterpene as a kairomone [17, 52] and other potential semiochemicals to locate their prey [8, 53]. In Sphaerophoria rueppelli, adult females are strongly attracted to odors from aphid colonies showing that specific volatile compounds are important to detect their prey [1]. Some studies on the relationships between aphid or host plant volatile emissions and aphid localization and foraging behavior have shown strong associations with syrphid recognition. One striking finding has shown that volatiles from plants attacked by aphids produce strong electrophysiological responses from the antennae of syrphids [7, 8]. These studies indicate that detecting prey-derived volatiles ((E)-β-farnesene), herbivore-induced plant volatiles (monoterpenes and sesquiterpenes), or naturally occurring general leaf volatiles (GLVs; alcohols, aldehydes and esters) help natural enemies to select oviposition sites and locate their prey [8, 12, 13, 17, 52–55].

Despite these reports on chemo sensory behavior, little is known on the molecular basis of syrphid olfaction. Therefore, the identification of predatory syrphid chemosensory gene families will help reveal how syrphids forage on their prey and choose oviposition sites. In this study we selected two syrphid species, E. balteatus and Eupeodes corollae Fabricius, active in northern China cotton fields to perform antennal transcriptome sequencing in order to explore and compare chemosensory genes in the two species. A total of 154 and 134 chemosensory candidate genes were identified in E. balteatus and E. corollae transcriptomes, respectively, including ORs, IRs, GRs, OBPs, CSPs and SNMPs. Furthermore, we report the expression profile of the OR families found in each insect transcriptome. A comparison between these two syrphids and other insect species revealed candidate chemosensory genes that could be involved in prey selection and plant volatile recognition. The discovery of putative chemosensory genes gives way for further exploration into functional assessments regarding chemoreception association.

Results
Antennal transcriptome sequencing and sequence assembly
E. balteatus and E. corollae antennal transcriptomes were sequenced using the Illumina HiSeq 2000 platform combined with Trinity assembly. Approximately 68.71 million and 77.28 million raw-reads were obtained from E. balteatus male and female antenna, respectively, reduced after filtering, to 65.69 and 74.25 million clean-reads. These were assembled into 57,950 unigenes for male and 68,165 for female. A final transcript dataset with 53,575 unigenes was obtained, consisting of 17,407 distinct clusters and 36,168 distinct singletons. The dataset was 47.61 megabase (Mb) in size with a mean length of 889 bp and N50 of 1724 bp (Additional file 1: Table S1). Parallel experiments generated 80.15 million and 77.38 million raw-reads in E. corollae male and female, respectively, and 65.69 and 74.25 million clean-reads. From these datasets 54,116 and 61,220 unigenes were obtained for male and female, respectively. The final
transcript dataset of *E. corollae* contained 50,942 unigenes with a mean length of 1039 bp and N50 length of 2104 bp, consisting of 18,054 distinct clusters and 32,888 distinct singletons (Additional file 1: Table S1). In addition, unigenes with a sequence length > 500 bp accounted for 47.64% and 43.59% of the *E. corollae* and *E. balteatus* transcriptome assembly, respectively.

### Homology analysis and gene ontology (GO) annotation

A BLASTX homology search against the NCBI non-redundant protein database indicated 23,680 (44.2%) and 25,606 (50.3%) unigenes from *E. balteatus* and *E. corollae*, respectively, with sequence similarities to known proteins using a cut-off E-value of $10^{-5}$. For *E. balteatus*, the larger number of similar genes (27.4%) belonged to *Ceratitis capitata* followed by *M. domestica* (20.4%), *D. melanogaster* (7.4%), *Drosophila willistoni* (3.1%), *Drosophila virilis* (3.0%) and *Drosophila mojavensis* (2.5%). For *E. corollae*, again *C. capitata* was best represented (25.1%), followed by *M. domestica* (18.8%), *D. melanogaster* (7.1%), *D. willistoni* (2.9%), *Acyrthosiphon pisum* (2.8%), and *D. virilis* (2.5%) (Additional file 2: Fig. S1A).

Gene ontology (GO) annotations were used to classify the transcripts into functional groups in accordance with specific GO categories. A total of 12,441 (23.22%) of all predicted proteins from *E. balteatus* and 12,425 (24.39%) predicted proteins from *E. corollae* were assigned to at least one GO term (Additional file 2: Fig. S1B). The GO terms distribution in the three categories were similar in the two species. In the “molecular function” category, the most abundant GO terms were “binding” and “catalytic activity”. In the “biological process” category, “cellular process”, “single-organism process” and “metabolic process” were the most represented. Finally, “cell”, “cell part”, and “organelle” were the most abundant GO terms in “cellular component” category (Additional file 2: Fig. S1B). GO terms associated with chemosensory genes were distributed in the “biological process” category (e.g. “cellular process”, “developmental process”, “response to stimulus”, “establishment of localization”, and “biological regulation”, etc.), “molecular function” category (e.g. “molecular transducer activity”, etc.) and “cellular component” category (e.g. “extracellular region”, “membrane part”, “membrane”, etc.).

### Candidate ORs in *E. balteatus* and *E. corollae*

Based on our analysis of the antennal transcriptomes in the two species, 51 and 42 transcripts for candidate ORs were identified in the combined male and female data sets from *E. balteatus* and *E. corollae*, respectively (Additional file 3: Table S2). A total of 21 *E. balteatus* ORs (EbalORs) and 29 *E. corollae* ORs (EcorORs) contained full-length open reading frames (ORFs), whose translation products are predicted to possess 2–8 transmembrane domains (TMDs). Other partial length transcripts encoded proteins exhibiting overlapping regions with low identity and were classified as unique genes. After a more exhaustive comparison with OR genes from other insect species, we found that all putative EbalORs shared between 22% and 86% amino acid identity with other ORs, with almost identical values (22% to 87%) for EcorORs. Detailed information is reported in Additional file 3 and Table S2.

We next performed a phylogenetic analysis using our candidate ORs and the ORs from four other Diptera species including *B. dorsalis*, *C. stygia*, *D. melanogaster* and *M. domestica* (Fig. 1). Clustered with DmOR83b, the highly conserved co-receptor Orco, orthologous genes were identified in the antennal transcriptomes of both syrphid species, and named EbalOrco and EcorOrco. As expected, sequence identity between EbalOrco and EcorOrco is very high (97.27%). Among the other ORs, five EbalORs (EbalOR9, 16, 18, 22 and 37) and three EcorORs (EcorOR8, 24 and 29) clustered with DmelOR67d, the pheromone receptor from *D. melanogaster*. This OR67d specific clade also included the OR67d orthologues from *M. domestica* and *B. dorsalis*. Two of these genes, EbalOR16 and EcorOR24, are full-length transcripts with 71.65% amino acid identity. The remaining ORs in this group were highly divergent among different species. Within the Dipteran OR sequences, we found a species-specific clade including eight members from *E. balteatus* (EbalOR7, 10, 30, 32, 41, 46, 47 and 48) and seven from *E. corollae* (EcorOR9, 10, 11, 15, 16, 34 and 38) that shared low identities with other Dipteran ORs (Fig. 1).

Amino acid identity between gene products correlates with similarity between genes. An amino acid sequence comparison between EbalORs and EcorORs revealed 33 pairs of orthologous ORs (including Orco) with 73.86% identity and the amino acid identity of complete OR ORFs was 84.00%. Sequence similarity percentages of the 28 pairs of homologous ORs are greater than 60% (Additional file 4: Table S3). In addition to the Orco family, orthologous groups with identities higher than 90% include the EbalOR29/EcorOR36, EbalOR3/EcorOR5, EbalOR13/EcorOR19 EcorOR22 with/EcorOR22 with 94.88%, 92.20%, 91.46% and 91.30% sequence identities, respectively. In addition, 10 EbalOR sequences (EbalOR2, 5, 7, 8, 11, 14, 21, 23, 25 and 33) are closely related to EcorOR homologues (EcorOR3, 4, 7, 9, 13, 14, 23, 28, 31 and 35) with identities greater than 80% (Additional file 4: Table S3; Fig. 1). All of these highly homologous proteins may play important roles in olfactory recognition.

### Candidate GRs in *E. balteatus* and *E. corollae*

We have identified 14 and 16 candidate GR genes from *E. balteatus* and *E. corollae* transcriptomes, respectively (Additional file 3: Table S2). The majority of candidate EbalGRs and EcorGRs were partial fragments, with only three from *E. balteatus* and six from *E. corollae* encoding full-length proteins. These complete sequences all show
six or seven TMDs with an intracellular N-terminus and extracellular C-terminus. Phylogenetic analysis with GRs from six Dipteran species suggest that *Drosophila* GR21a and GR63a, reported as carbon dioxide sensors [42, 56], clustered first with EbalGR2 and EcorGR2 and second with EbalGR1 and EcorGR1. In addition, EbalGR4, EbalGR13 and EcorGR4 showed high identities to thermoreceptor DmelGR28b responsible for rapid warmth avoidance [57].

Several other GRs clustered with members of candidate sugar detection GR (GR5a, GR61a and GR64a-f) subfamily (Fig. 2) [58–62].

**Candidate IRs in *E. balteatus* and *E. corollae***

We identified 32 transcripts for putative ionotropic receptors in *E. balteatus* and 23 in *E. corollae*. Of these, seven EbalIRs and 14 EcorIRs contained full-length ORFs, with
two to five TMDs (Additional file 3: Table S2). Among these we found the common conserved co-receptors IR8a (EbalIR8a and EcorIR8a) and IR25a (EbalIR25a and EcorIR25a) in both species. Other candidate IRs were found as partial sequences (Fig. 3).

In order to further distinguish putative IRs from iGluRs, all EbalIRs and EcorIRs were aligned with IRs from *A. gambiae*, *C. stygia* and *D. melanogaster*, as well as some AgamiGluRs and DmeliGluRs for phylogenetic analysis. The results showed that the candidate EbalIRs and EcorIRs clustered with presumed “antennal” orthologues IR76b, IR93a, IR21a, IR68a, IR40a, IR75l, IR75d, IR64a, IR84a, IR31a and IR92a, and were well separated from the AgamiGluRs and DmeliGluRs clade (Fig. 3) [63]. Interestingly, the conserved “antennal” orthologues, IR60a, was lacking from *E. balteatus* and *E. corollae* transcriptome assemblies, while IR68a was only absent from *E. corollae*. The sequences of *E. balteatus* clustering with DmellIR94d and DmellIR94e were quite divergent (Fig. 3; Additional file 5: Fig. S2). When compared to the orthologues within other species, these IRs may play different roles in olfaction.

**Candidate OBPs in *E. balteatus* and *E. corollae***

We identified 49 different transcripts encoding candidate OBPs in *E. balteatus* and 44 in *E. corollae*, numbers similar to the 52 OBPs of *D. melanogaster* [64]. Of these, 38 transcripts of EbalOBPs and 31 EcorOBPs contained full-length ORFs with predicted signal peptide sequences (with EbalOBP31 as the only exception) (Additional file 3: Table S2).

A phylogenetic tree was built with these sequences and those of orthologous from *B. dorsalis*, *C. stygia*, *D. melanogaster* and *M. domestica*. Among EbalOBPs, thirty-one showed the classic motif of six conserved cysteines, three were Plus-C (EbalOBP2, 3, 4) and fifteen were Minus-C (EbalOBP5, 7, 8, 9, 11, 12, 14, 15, 16, 18, 21, 25, 26, 44 and 45) (Fig. 4). For EcorOBPs, we found 30 classic, 5 Plus-C
(EcorOBP1, 3, 4, 5, 41) and 9 Minus-C (EcorOBP6, 7, 8, 10, 11, 13, 18, 36 and 37) (Fig. 4) [64, 65]. One large group of classic OBPs, including 15 EbalOBPs and 14 EcorOBPs showed large differences compared to sequences of other species and could represent OBPs specific of syrphids (Fig. 4; Additional file 6: Fig. S3). We found the orthologue of DmelOBP-lush in both species, EbalOBP17 and EcorOBP14. These two proteins are 98.64% identical at the amino acid level between each other and 39.87% and 40.52% identity to DmelOBP-lush.

Candidate CSPs in *E. balteatus* and *E. corollae*

Through bioinformatic analysis, six and seven different transcripts encoding candidate CSPs were identified from *E. balteatus* and *E. corollae* transcriptomes, respectively. Five EbalCSPs and six EcorCSPs represented full-length proteins and only EbalCSP6 lacked a signal peptide (Additional file 3: Table S2). All of the identified amino acid sequences possessed the highly conserved four-cysteine profile. A phylogenetic tree was built with all the syrphid CSPs and those of *A. gambiae*, *C. stygia*, *D. melanogaster* (Fig. 5).

Candidate SNMPs in *E. balteatus* and *E. corollae*

In both species, two SNMPs with full-length ORFs were identified possessing two TMDs (with EbalSNMP1 having a single TMD as an exception) (Additional file 3: Table S2). EbalSNMP1 and EcorSNMP1 are very similar to DmelSNMP1, a protein shown to be required for correct pheromone detection [50, 66–68]. EbalSNMP2 and EcorSNMP2 are similar to DmelSNMP2, reported to be expressed in supporting cells (Fig. 6) [27, 69, 70].

Differentially expressed genes (DEGs) analysis

Gene expression levels of all male and female antennae-associated chemosensory genes in both *E. balteatus* and *E. corollae* were assessed using fragments per kilobase per million fragments (FPKM) values, represented in a heatmap (Fig. 7). Normalised antennal expression levels of candidate *E. balteatus* and *E. corollae* ORs are shown in Additional file 7. Of all ORs, Orco had the highest expression level of transcripts in both sexes of each species. There were no significant differences of OR transcript abundances (FPKM value) in the respective male and female antenna, except for EcorOR14 (Additional file 7). A combined analysis of
false discovery rate (FDR) ≤0.001 and |log2 Ratio| ≥ 1 showed that EcorOBP shared highest number of differentially expressed genes (DEGs), including eleven high-expression in male and six high-expression in female syrphids (Additional file 7). In addition, candidate carbon dioxide receptor GR1 and GR2, and SNMP1 in both sexes showed a high expression level (Fig. 7).

**Tissue- and sex- specific expression of candidate E. balteatus and E. corollae OR genes**

The expression of the candidate ORs in *E. balteatus* and *E. corollae* male and female antennae and legs (control sample) was analyzed using semi-quantitative reverse transcription PCR (RT-PCR). All 51 EbalORs and 42 EcorORs were detected in the antennae at high expressing level. Only EbalOR49 was found to be mainly expressed in legs. There were no significant differences of transcript abundances in the respective male and female antenna (Fig. 8). The Orco co-receptor gene also showed a high expression level in both syrphid species. This is consistent with DEGs analysis of OR transcript abundances using the FPKM value.

**Discussion**

The syrphids *E. balteatus* and *E. corollae* are aphid-specific predators and predominately inhabit northern China wheat and cotton fields. Typical of most insects, chemical cues drive several aspects of their behavior, such as foraging on prey and choosing oviposition sites [7, 8, 10]. Chemosensory proteins play an important role in this process. We analyzed antennal transcriptomes of *E. balteatus* and *E. corollae* and searched for chemosensory genes with the purpose of understanding chemical communication of tritrophic interactions among plants, herbivorous insects, and natural enemies.

In our study, we sequenced *E. balteatus* and *E. corollae* antennal transcriptomes using next generation sequencing technology on the Illumina HiSeq 2000 platform. The total RNA was converted into a template library for high throughput DNA sequencing, allowing
Fig. 5 Phylogenetic tree of candidate *E. balteatus* and *E. corollae* CSPs and other Dipteran CSPs. The distance tree was rooted by AgamCSP1/2/3 genes. Bootstrap values are shown. The species used to construct tree include *E. balteatus* (Ebal, red), *E. corollae* (Ecor, blue), *D. melanogaster* (Dmel, dark), *A. gambiae* (Agam, green), and *C. stygia* (Csty, magenta).

Fig. 6 Phylogenetic tree of candidate *E. balteatus* and *E. corollae* SNMP and other Dipteran SNMP. Bootstrap values are shown. The species used to construct tree including *E. balteatus* (Ebal, red), *E. corollae* (Ecor, blue), *D. melanogaster* (Dmel, dark) and *A. gambiae* (Agam, green).
Fig. 7  Expression profiles of chemosensory genes in *E. balteatus* and *E. corollae*. a: CSPs; b: GRs; c: IRs; d: ORs; e: OBPs; and f: SNMPs

Fig. 8  Tissue- and sex-specific expression of candidate *E. balteatus* and *E. corollae* OR genes. M: male antennae, F: female antennae, L: legs (both sexes mixed)
us to obtain all expressed transcripts. De novo assembly of transcripts using the Trinity method gives high-efficiency and reliable full-length transcripts across extensive expression levels, even without genome information [71]. Our sequence assembly yielded a final transcript dataset of 50,942 unigenes from *E. corollae* and 53,575 *E. balteatus* unigenes. Total unigenes counts resulted in 44.2% unigenes from *E. balteatus* and 50.3% unigenes from *E. corollae* shared sequence similarities to known proteins using the BLASTX homology search from the NCBI non-redundant protein database. These percentages are very similar to other Dipteran species [50, 72]. Remaining transcripts without associated GO terms may represent species-specific genes. The antennal transcriptome analysis proved to be a powerful tool to identify chemosensory genes in insects without genome information. It has been successfully employed in many insect orders including Lepidoptera, Coleoptera, Hymenoptera and Hemiptera. In Diptera, the chemosensory genes were successfully identified in *C. stygia*, *B. dorsalis* and *Scaeva pyrastri* antennal transcriptomes [50, 72, 73]. Here, we identified 154 and 134 candidate chemosensory genes in *E. balteatus* and *E. corollae*, respectively, a number similar to other Diptera antennal transcriptomes (e.g. 128 in *C. stygia*) [50] but less than the chemosensory genes identified in *D. melanogaster* (254), *M. domestica* (386) and *A. gambiae* (292) genome [30, 33, 47, 49, 63, 65, 74]. This could be the result of differential expression based on developmental stages of the insect larva or adult olfactory organ development such as maxillary palp and proboscis. All data shows that the chemosensory genes identified by antennal transcriptome sequencing are accurate and reliable.

We identified 154 candidate chemosensory genes (51 ORs, 32 IRs, 14 GRs, 49 OBPs, 6 CSPs and 2 SNMPs) in *E. balteatus* and 134 (42 ORs, 23 IRs, 16 GRs, 44 OBPs, 7 CSPs and 2 SNMPs) in *E. corollae*, numbers slightly different compared with those of other Dipteran species [50, 73, 75, 76]. Such differences could be due to sequencing methods, coverage and/or depth. The number of chemosensory genes is higher in *E. balteatus* than in *E. corollae*. However, assembling and splicing quality (unigene number and N50 length) in *E. corollae* is better than in *E. balteatus*. The differences in the number and quality of transcripts identified could arise from variations in sample preparation or could be due to evolution [77] and adaptation to the environment (tritrophic interactions).

A total of 49 and 44 OBPs were identified in *E. balteatus* and *E. corollae* transcriptomes, respectively. The number of OBPs is variable across species, with 52 members in *D. melanogaster*, 66 in *A. gambiae* (Diptera), 21 in *Apis mellifera* (Hymenoptera), 34 in *Helicoverpa armigera*, 29 in *Helicoverpa assulta* (Lepidoptera), 26 in *Colaphellus bowringi*, 46 in *Tribolium castaneum* (Coleoptera) and 15 in *A. pisum* (Hemiptera) [27, 74, 77–83]. Meanwhile, the OBPs of these two syrphid species are highly divergent with those of other insects. These evolutionary differences may result from different physiological functions or ecological niches. Compared with OBPs, only a small amount of CSPs were detected in Diptera. They are only 4 CSPs in *D. melanogaster*, 4 in *C. stygia* and 7 in *A. gambiae* (Diptera). These numbers are much lower than in other insect orders, such as 18 CSPs found in *H. armigera* and 17 in *H. assulta* (Lepidoptera) [27, 50, 80, 81]. In our study, six EbalCSPs and seven EcorCSPs are identified in transcriptome sequencing, revealing that the numbers of CSP gene family differ among species. CSPs show a high evolutionary diversity in Inseta, probably related to different physiological functions.

We identified 51 ORs from *E. balteatus* and 42 ORs from *E. corollae*, respectively. Compared with other Dipteran species, these numbers are similar to those identified in *C. stygia* (50) [50] and *G. morsitans* morsitans (46) [51] but lower than those of *D. melanogaster* (62), *M. domestica* (86), *A. gambiae* (79) [47, 49, 84–86], suggesting that sequencing method/depth may be different between studies yielding less genes that may be difficult to detect because of low expression [77]. Here, we were able to detect species-specific OR transcripts in *E. balteatus* and *E. corollae*. This clade of ORs may have a greater impact on recognizing specific odors, particularly perception of aphids-derived volatiles and herbivore-induced plant volatiles granting syrphid localization access of its prey.

The tissue- and sex-specific expression analysis showed no differences between male and female, which is consistent with DEGs analysis of OR transcript abundances using FPKM values. Lepidopteran ORs have shown male-specific expression that is usually involved in the detection of the sex pheromone [19, 26, 27], but this does not seem to be the case in syrphids. Additional real time quantitative PCR, in situ hybridization and single-sensilla recordings would be required to validate OR expressions and functions.

In *D. melanogaster*, males release the volatile sex pheromone cis-vaccenyl acetate (cVA) [87–89]. The perception of sex pheromone cVA is mediated by OR67d [88], OR65a [90, 91], LUSH [92], and SNMP1 [34]. In our two syrphid species, EbalOBP17 and EcorOBP14 are the orthologues of the DmelOBP-lush gene, while EbalOR16 and EcorOR24 are the orthologues of DmelOR67d, and EbalSNMP1 and EcorSNMP1 are very similar to DmelSNMP1, suggesting that these proteins may be involved in detection of their yet unidentified pheromones. Therefore, further functional characterization of these candidate proteins will help reveal any mechanism associated with pheromone reception in *E. balteatus* and *E. corollae*.

The *E. balteatus* and *E. corollae* IR family is relatively conserved, especially with respect to common receptors IR8a and IR25a, which are expressed in both olfactory and
gustatory systems [30, 45]. The numbers of IRs identified in *E. balteatus* (32) and *E. corollae* (23) are similar to that of *C. stygia* (22) [50], but lower than those of *D. melanogaster* (66) and *A. gambiae* (46) [63]. It is possible that some IRs do not express in antennae tissues or perhaps the number of IRs varies between species and is dependent on natural habitats. A large number of EbalIRs and EcorIRs are clustered with “antennal” orthologues in *Drosophila*, indicating that IRs are highly conserved in Diptera. Furthermore, the IRs identified in these two species may be activated by acids, amines and other odorants that are not sensed by ORs [30, 45, 46].

In the antennae of *E. balteatus* and *E. corollae*, we identified 14 and 16 candidate GRs, respectively. The total number of GRs in these two species may be much larger, because some members could be exclusively expressed in other gustatory organs, such as maxillary palps, proboscides and legs. However, the numbers are still lower than those reported in other Dipteran antennal transcriptomes [50]. The conserved receptors identified in the two syrphid species may be involved in CO2 perception. However, we infer that the mechanism of CO2 perception is different from mosquitoes which concerns host-seeking [43, 44, 48, 93]. Some GRs may function as taste or contact receptors [31], particularly with reference to their specific pollination behavior [94, 95]. Some GRs from these two species are clustered with thermos-sensing GRs and sugar-detecting GRs from *Drosophila*, indicating that they may perform similar functions. Functional analysis of the candidate *E. balteatus* and *E. corollae* chemosensory proteins is required to identify their physiological roles.

**Conclusions**

We have identified and annotated 154 transcripts encoding putative chemosensory proteins in antennal transcriptome of *E. balteatus* and 134 in *E. corollae*. Comparisons between the two syrphid species and among other Dipteran species were deduced using sequence information. This work gives a foundation for future studies aimed at understanding chemical communication in syrphids and tritrophic interactions between plants, herbivorous insects, and natural enemies in agricultural ecosystems.

**Methods**

**Insect rearing and tissue collection**

*E. balteatus* and *E. corollae* larvae were fed with aphids (*Aphis gossypii* Glover) and maintained at 22 ± 1 °C with a 12 h light: 12 h dark photo-period at the Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, China. Following eclosion, adult males and females were separated and provided with pollen and 10% honey solution.

Antennae were excised from 2- to 5-day-old adult males and females respectively, and legs were collected together, then immediately frozen and stored in liquid nitrogen.

**cDNA library construction and Illumina sequencing**

Total RNA of male and female antennae was extracted from *E. balteatus* and *E. corollae* using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). The method for RNA extraction followed in the manufacturer’s instruction. Total RNA was dissolved in RNase-free water and RNA integrity was verified by gel electrophoresis. RNA concentration and purity were measured on a Nanodrop ND-2000 spectrophotometer (Nanodrop products, Wilmington, DE, USA). Ten micrograms total RNA of each sample was used to construct the cDNA library. The cDNA library construction and Illumina HiSeq 2000 (Illumina, San Diego, CA, USA) sequencing of the samples was performed at Beijing Genomics Institute (BGI, Shenzhen, China). The insert sequence length was around 200 bp and these libraries were pair-end sequenced using PE100 strategy [22, 27].

**Assembly and function annotation**

Raw reads were pre-processed by filtering low quality reads, trimming low quality nucleotides at each ends and removing 3′ adaptors and poly-A/T tails. Each clean-read dataset of male and female antenna was fed to Trinity [71]. The Trinity assembly procedure, including Inchworm, Chrysalis and Butterfly were followed using Grabherr *et al.*, 2011 as a reference [71]. In the first step of Trinity, Inchworm assembles reads into the unique sequences of transcripts using the default parameters (default k-mers = 25). Next, Chrysalis clusters related contigs that correspond to portions of alternatively spliced transcripts or otherwise unique portions of paralogous genes. Finally, Butterfly uses read sequences, read-pairings and Chrysalis’ read mappings to select the paths that are best supported by read sequences [71].

The Trinity outputs were clustered by TGICL [96]. The consensus cluster sequences and singletons make up the unigenes dataset [22]. The unigenes annotation was performed by NCBI BLASTX against a pooled database of non-redundant and SwissProt protein sequences with e-value <1e-5. The BLASTX results were then imported into Blast2GO pipeline for GO annotation [97].

**Identification of chemosensory genes**

Candidate unigenes encoding putative ORs, IRs, OBPs, CSPs, SNMPs and GRs were found by running Perl scripts against transcriptome assembly and annotation in the remote server. Perl scripts were written to extract sequence from functional annotation results using olfaction keywords. Subsequently, all candidate chemosensory genes were manually checked by BLASTX against local non-redundant database with e-value <1e-5. Using the BLASTX NCBI database, we manually performed alignments comparing transcripts against all known
proteins to examine full-length coverage. The full-length transcripts contain start and termination codons. The ORFs of all putative chemosensory genes were predicted by using ExPaSy (Expert Protein Analysis System) server version (http://web.expasy.org/translate/) according to the BLASTX best hit result [98]. Putative N-terminal signal peptide of OBPs and CSPs were predicted by SignalP 4.0 server version with default parameters [99]. The TMDs of ORs, IRs and GRs were predicted using TMHMM server version 2.0 [100].

**Sequence and phylogenetic analysis**

After removing redundancy, alignments of amino acid sequences were performed by MAFFT (https://www.ebi.ac.uk/Tools/msa/mafft/). The phylogenetic trees of *E. balteatus* and *E. corollae* chemosensory genes were constructed by RAxML version 8 with Jones-Taylor-Thornton amino acid substitution model (JTT) [101] with the putative chemosensory genes in other Dipteran species (Additional file 8: Table S4). Node support was assessed using a bootstrap method based on 1000 replicates. The OR data set contained OR sequences identified in Dipteran (51 from *E. balteatus*, 42 from *E. corollae*, 62 from *D. melanogaster* [85, 86], 61 from *B. dorsalis* [genome: assembly ASM78921v2], 50 from *C. stygia* [50] and 81 from *M. domestica* [49]). The GR data set contained GR sequences identified in Dipteran (14 from *E. balteatus*, 16 from *E. corollae*, 68 from *D. melanogaster* [47], 40 from *B. dorsalis* [genome: assembly ASM78921v2], 21 from *C. stygia* [50] and 43 from *M. domestica* [49]). The IR data set contained IR sequences identified in Dipteran (32 from *E. balteatus*, 23 from *E. corollae*, 76 from *D. melanogaster* [30, 63], 22 from *C. stygia* [50] and 54 from *A. gambiae* [63, 84]). The OBP data set contained OBP sequences identified in Dipteran (49 from *E. balteatus*, 44 from *E. corollae*, 71 from *D. melanogaster* [64], 40 from *B. dorsalis* [genome: assembly ASM78921v2], 28 from *C. stygia* [50] and 52 from *M. domestica* [49]). The CSP data set contained CSP sequences identified in Dipteran (7 from *E. balteatus*, 9 from *E. corollae*, 4 from *D. melanogaster* [74], 4 from *C. stygia* [50] and 8 from *A. gambiae* [74]). The SNMP data set contained SNMP sequences identified in Dipteran (2 from *E. balteatus*, 2 from *E. corollae*, 2 from *D. melanogaster* [34, 35] and 2 from *A. gambiae* [35]).

**DEGs analysis**

A mapping-based expression profiling analysis of the chemosensory genes was conducted to compare gene expression between male and female antennae. All of the clean reads were remapped onto the transcripts using SOAPaligner (http://soap.genomics.org.cn/soapaligner.html), allowing up to three base mismatches and a minimum length of 40 bp. The FPKM method was used for calculating unigene expression levels [20, 50, 102, 103]. The suitable *P*-values were calculated to identify differentially expressed genes according to the hypergeometric test [103]. The FDR was a statistical method used in multiple hypothesis testing to correct for *P*-value. Criteria for estimating significant differential expression was set at FDR ≤ 0.001 and |log2 Ratio| ≥ 1. Heatmaps of differential gene expression between male antennae and female antennae in both species were generated by Heml 1.0 software [104].

**Expression analysis by semi-quantitative RT-PCR**

Semi-quantitative RT-PCR was performed to verify the expression of candidate chemosensory genes. Male and female antennae and legs were collected from adult *E. balteatus* and *E. corollae* after eclosion. The extraction of total RNA followed the manufacturer’s instruction [27]. The cDNA was synthesized from total RNA using ReverTra Aid First Strand cDNA Synthesis Kit (Thermo Scientific, Waltham, MA, USA). Gene specific primers were designed using PrimerQuest Tool (http://sg.idtdna.com/Primerquest/Home/Index) (Additional file 9: Table S5) and synthesized by Sangon Biotech Co., Ltd. (Shanghai, China). A Taq MasterMix (CW BIO, Beijing, China) was used for PCR reactions under the general three-step amplification of 94 °C for 30s, 55 °C for 30s, 72 °C for 30s. RTPCR products were separated on 2% agarose gels, stained by ethidium bromide (EB), and photographed under UV light in Gel Doc XR+ Gel Documentation System with Image Lab Software (Bio-Rad, Hercules, CA, USA).

**Additional files**

Additional file 1: Table S1. Assembly summary of *E. balteatus* and *E. corollae* antennal transcriptome. (DOCX 16 kb)

Additional file 2: Fig. S1. (A) Species distribution and annotation summaries in the *E. balteatus* (Ebal) and *E. corollae* (Ecor) antennal transcriptome assembly. (B) Gene ontology classifications of the *E. balteatus* and *E. corollae* unigenes with Blast2GO program, including categories with biological process, molecular function and cellular component. (TIFF 3397 kb)

Additional file 3: Table S2. Candidate *E. balteatus* and *E. corollae* antennal chemosensory genes. Unigenes of candidate odorant receptors (2–1), gustatory receptors (2–2), ionotropic receptors (2–3), odorant binding proteins (2–4), chemosensory proteins (2–5) and sensory neuron membrane proteins (2–6) with gene name, length, ORF, best BLASTX hit and identity. (DOCX 112 kb)

Additional file 4: Table S3. Comparison of homologous ORs in *E. balteatus* and *E. corollae*. (DOCX 61 kb)

Additional file 5: Fig. S2. Protein domain analysis of the species-specific IR clade with Drosophila IgIURs and DmelIR94d /e. Amino acid alignments shows the ligand binding domains (S1 and S2), the ion channel pore (P), and TMD (M1, M2 and M3) of ionotropic receptors. The key ligand binding residues are marked in red box. (JPG 4154 kb)

Additional file 6: Fig. S3. Protein domain analysis of the species-specific IR clade with Drosophila IgIURs and DmelIR94d /e. Amino acid alignments shows the ligand binding domains (S1 and S2), the ion channel pore (P), and TMD (M1, M2 and M3) of ionotropic receptors. The key ligand binding residues are marked in red box. (JPG 4154 kb)

Additional file 7: Table S4. GenBank accession numbers of chemosensory genes used in phylogenetic analyses. (XLSX 34 kb)

Additional file 8: Table S5. Primers of candidate ORs in *E. balteatus* and *E. corollae* used for RT-PCR. (DOCX 20 kb)
Abbreviations
cDNA: Complementary DNA; CSP: Chemosensory protein; DEGs: Differentially expressed genes; FDR: False discovery rate; FPKM: Fragments per kilobase per million fragments; GLV: Green leaf volatiles; GO: Gene ontology; GR: Gustatory receptor; IR: Ionotonic receptor; OBP: Odorant-binding protein; OR: Odorant receptor; ORF: Open reading frame; PCR: Polymerase chain reaction; RT-PCR: Semi-quantitative reverse transcription PCR; SNMP: Sensory neuron membrane protein; TMD: Transmembrane domain

Acknowledgements
We thank Ph.D. students Kang He, Song Cao, Rui-Bin Zhang and M.S. student Yan-Wei Wang (Institute of Plant Protection, Chinese Academy of Agricultural Sciences) for help collecting insects. We thank Ms. Chun-Yan Wang and Ms. Li-Yan Yang (Institute of Plant Protection, Chinese Academy of Agricultural Sciences) for rearing insects. We are grateful to thank M.S. Xiang-Zhi Liang, Dr. Yong-Lei Liu and Dr. Ya-Nan Zhang for technical assistance. Thanks to Prof. Paolo Pelosi for editorial assistance and comments on the manuscript.

Funding
This project is supported by National Natural Science Foundation of China (311621004, 3120062831, 3210042 to BW, 31,402023 to BW, 31,471833 to YL), China Postdoctoral Science Foundation (2014MS50095 to BW), China Postdoctoral Science Foundation 2017T100121 to BW.

Availability of data and materials
The clean reads of the four antennal transcriptomes in this study have been stored in the NCBI SRA database, under the accession number of SRX1812754 (E. balleatus male), SRX1812787 (E. balleatus female), SRX1829150 (E. coralloid male), and SRX1829282 (E. coralloid female).

Authors’ contributions
All authors contributed to research design and manuscript preparation. Conceived and designed the experiments: BW, YL, GW. Performed the experiments: BW. Analyzed the data: BW, YL. Contributed reagents/materials/analysis tools: BW, YL, GW. Wrote the paper: BW, YL, GW. All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

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Received: 24 January 2017 Accepted: 12 July 2017
Published online: 07 August 2017

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