Identification of differentially expressed genes based on antennae RNA-seq analyses in Culex quinquefasciatus and Culex pipiens molestus

Heting Gao†, Zhenyu Gu†, Dan Xing†, Qiaojiang Yang, Jianhang Li, Xinyu Zhou, Teng Zhao* and Chunxiao Li*

Abstract

Background: Both Culex quinquefasciatus and Cx. pipiens molestus are sibling species within Cx. pipiens complex. Even though they are hard to distinguish morphologically, they have different physiological behaviors. However, the molecular mechanisms underlying these differences remain poorly understood.

Methods: Transcriptome sequencing was conducted on antennae of two sibling species. The identification of the differentially expressed genes (DEGs) was performed by the software DESeq2. Database for Annotation, Visualization and Integrated Discovery was used to perform GO pathway enrichment analysis. The protein–protein interaction (PPI) network was constructed with Cytoscape software. The hub genes were screened by the CytoHubba plugin and Degree algorithms. The identified genes were verified by quantitative real-time PCR.

Results: Most annotated transcripts (14,687/16,005) were expressed in both sibling species. Among 15 identified odorant-related DEGs, OBP10 was expressed 17.17 fold higher in Cx. pipiens molestus than Cx. quinquefasciatus. Eighteen resistance-related DEGs were identified, including 15 from CYP gene family and three from acetylcholinesterase, in which CYP4d1 was 86.59 fold more highly expressed in C. quinquefasciatus. Three reproductive DEGs were indentified with the expression from 5.01 to 6.55 fold. Among eight vision-related DEGs, retinoic acid receptor RXR-gamma in Cx. pipiens molestus group was more expressed with 214.08 fold. Among the 30 hub genes, there are 10 olfactory-related DEGs, 16 resistance-related DEGs, and four vision-related DEGs, with the highest score hub genes being OBP lush (6041148), CYP4C21 (6044704), and Rdh12 (6043932). The RT-qPCR results were consistent with the transcriptomic data with the correlation coefficient R = 0.78.

Conclusion: The study provided clues that antennae might play special roles in reproduction, drug resistance, and vision, not only the traditional olfactory function. OBP lush, CYP4C21, and Rdh12 may be key hints to the potential molecular mechanisms behind the two sibling species' biological differences.

Keywords: Mosquitoes, Antennae, Olfactory, Reproduction, Resistance, Vision, RNA-seq

© The Author(s) 2022. Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated in a credit line to the data.
Culex mosquitoes are vectors of West Nile virus, Japanese encephalitis virus, and lymphatic filariasis [3].

Culex quinquefasciatus (Cx. quinquefasciatus, Cqui) and Cx. pipiens molestus (Cx. p. molestus, Cmls) are sibling species within Cx. pipiens complex whose morphologies are difficult to distinguish [4]. However, Cx. p. molestus and Cx. quinquefasciatus have very different physiological behaviors. For example, Cx. p. molestus mainly mate in confined spaces. Their ovaries can develop normally, and they lay eggs without blood-feeding in their first life cycle. On the other hand, Cx. quinquefasciatus mate in open areas. There are also differences in their preference for mammalian and bird blood sources [5]. Although the different blood-feeding habits and other physiological behaviors of the two sibling species have been observed for a long time, their possible molecular mechanisms have still not been totally illustrated.

Antenna is the main chemosensory organ of mosquito and plays an important role in smell [6], hearing [7], host-locating [8], and courtship [9]. The mosquito antenna comprises three parts: scape, pedicel, and flagellum. The antenna and maxillary palp detect odors emanating from the host. With the help of proboscis and eyes, which detect taste and visual cues, mosquitoes are successful in flight navigation toward the host [10]. The expressions of many chemoreceptor genes have been found in the antennae of Anopheles sinensis, and their expression levels are significantly regulated after the blood meal [11], quite similar to the antennae of the Culex pipiens complex [12] and Aedes aegypti [13–15]. Besides the typical olfactory function, the antennae of mosquitoes are model systems for other sensations, including acoustics [16].

We conducted transcriptome sequencing on antennae of Cx. p. molestus and Cx. quinquefasciatus and then explored the differences in the expression and interaction of transcripts. Besides olfaction, antennae could also have special roles in reproduction, drug resistance, and visual function. The study provides hints about the potential molecular mechanisms behind the two sibling species’ biological differences.

**Methods**

**Mosquitoes**

Both Cx. p. molestus and Cx. quinquefasciatus were obtained from long-term laboratory-reared strains, which had been characterized by male genitalia and/or cytochrome oxidase subunit I (COI) barcoding in advance [17, 18]. The breeding conditions were as follows: temperature 26 ± 1 °C, relative humidity 70 ± 5%, and a light:dark regime 14 h:10 h.

**RNA extraction and library construction**

Two groups of samples were designed in this study: antennae of Cx. quinquefasciatus (Cqui) and antennae of Cx. p. molestus (Cmls). Biological samples containing 50 antennae were set in four replicates for each group. RNA was extracted with TRIzol (Takara.9108). Libraries were constructed using the NEB Next Ultra RNA Library Prep Kit and finally sent to Beijing Macro-Micro-test Biotechnology Co., Ltd., for transcriptome sequencing.

**Transcriptome sequencing analysis**

After the raw data were filtered, clean reads were compared to the reference genome using HISAT2 software [19]. Reference genome file was acquired from the data in the National Center for Biotechnology Information (NCBI)(GCF_015732765.1) [20, 21]. Transcript assembly was performed with StringTie software [22] followed by annotation from databases such as P fam [23], Gene Ontology (GO) [24, 25], and the Kyoto Encyclopedia of Genes and Genomes (KEGG) [26].

A differential analysis was performed using DESeq2 software [27]. The screening criteria for differentially expressed genes (DEGs) in antennae were $p$adj < 0.05 and $|\log2(foldchange)| > 1$. Functional annotation and GO enrichment analyses were carried out using the R and clusterProfiler [28, 29] to categorize the DEGs into biological process (BP), molecular function (MF), and cellular components (CC).

Protein-protein interaction (PPI) network analysis obtained the interaction network file through the STRING database [30]. The files were displayed using Cytoscape software [31]. The cytoHubba plug-in Degree topology algorithm was used to predict and explore to calculate gene scores for hub genes [32].

**qPCR verification of identified DEGs**

Ten genes with relatively high expressions and significant differences were randomly selected and combined with housekeeping genes 18S ribosomal RNA (18S) to verify the accuracy of the transcriptome results. The primers were designed with Oligo Primer Analysis software version 4.0 (Additional file 1: Table S1). A qPCR analysis was conducted with a One Step SYBR PrimeScript RT-PCR Kit II (Cat# RR086A, Takara). The reaction conditions were set as follows: 94 °C for 30 s, 94 °C for 5 s, and 60 °C for 30 s, repeated for 40 cycles. Three technical replicates were performed for each sample. The $2^{−ΔΔCT}$ method was applied to calculate the relative gene expression [33, 34]. A chi-square test was used to confirm the pairwise differences at the significance level of $α = 0.05$. The correlation
between RNA-seq and RT-qPCR expression was calculated by Pearson correlation.

**Result**

**Sequencing data quality**

After sample data were filtered and the adapter removed, the metagenomic sequencing depth of eight samples was around 30G. The number of valid reads ranged from 22876221 to 31754347. There were 206232097 reads (16,005 annotated transcripts) in total. The Q20 values were all > 95%, suggesting a high degree of quality (Table 1).

**Transcriptome differential expression analysis of antennae**

Principal component analysis showed that the ellipse represented the grouping area with 68% confidence interval; all biological replicates of Cqui and Cmls sample were distributed in two distinct groups (Fig. 1A). Most annotated transcripts (14,687/16,005) were expressed in both sibling species. The number of Cx. quinquefasciatus- and Cx. p. molestus-specific transcripts were 890 and 428, respectively (Fig. 1B). There were 1577 DEGs in the antennal transcriptome of Cqui and Cmls, of which 1166 DEGs were more highly expressed in the Cqui group and 411 DEGs were higher in the Cmls group (Fig. 1C).

With the purpose of better understanding the differences in regulation between the two sibling mosquito species’ antennae, we mapped all transcripts to GO pathways to exploit the pathways that were significantly enriched. DEGs with higher expression in the Cqui group were enriched in signal transduction, cell communication in BP; channel complex and neural synapse in CC; signaling receptor activity and channel activity in MF (Fig. 1D). DEGs with higher expression in the Cmls group were enriched in defense response, carbohydrate metabolic process in BP; cell projection, component of endoplasmic reticulum membrane in CC; oxidoreductase activity and ion binding pathways in MF (Fig. 1E).

**Specific functional DEGs in antennal transcriptome**

By further mining the data, we focused on four types of specific functional DEGs, which would affect the key physiological behaviors of mosquitoes, including olfactory, resistance, reproduction, and vision. A total of 15 odorant-related DEGs were identified, of which 11 were more highly expressed in the Cqui group and four more highly expressed in the Cmls group. Most (12/15) were odorant-binding proteins (OBP); two other DEGs (OR67d and OR7a) were odorant receptors (OR) and one (GR22) a gustatory receptor (GR). Of these, OBP10 in cmls groups were specially more highly expressed at 17.17 fold times compared with the Cqui group (Fig. 2A, Additional file 1: Table S2).

A total of 18 resistance-related DEGs were identified, including 15 from cytochrome P450 (CYP) gene family and three from acetylcholinesterase (AchE). Eight CYP family DEGs were more expressed in Cqui; for example, CYP4D1 was 86.59 fold more highly expressed. Three AchE genes were differentially expressed with fold change ranging from 0.39–4.59 (Fig. 2B, Additional file 1: Table S3).

Two related vitellogenin-A1 (Vg-A1) and one location of vulva defective 1 (lov-1) DEGs were more expressed in the Cmls, with expression from 5.01 to 6.55 fold. These three genes were all related to insect mating (Fig. 2C, Additional file 1: Table S4).

Four vision-related genes were more expressed in the Cqui group and four more expressed in the Cmls group. Typically, retinoic acid receptor (RAR) RXR-gamma in Cmls group was more expressed at 214.08 fold. Vertebrate ancient (VA) opsins in Cqui group was more expressed with 12.43 fold change (Fig. 2D, Additional file 1: Table S5).

---

**Table 1** Transcriptome sequencing data mapping summary

| Sample   | Reads number | Alignment rate (%) | Q20 (%) | GC (%) |
|----------|--------------|--------------------|---------|--------|
| Cmls_1   | 25985588     | 58.55              | 97.22   | 53.16  |
| Cmls_2   | 24538832     | 57.97              | 96.82   | 52.32  |
| Cmls_3   | 30471828     | 60.04              | 96.1    | 53.74  |
| Cmls_4   | 31754347     | 59.27              | 95.57   | 53.86  |
| Cqui_1   | 23234291     | 57.02              | 95.58   | 53.9   |
| Cqui_2   | 23513630     | 57.20              | 95.1    | 52.61  |
| Cqui_3   | 23857360     | 59.69              | 95.41   | 53.01  |
| Cqui_4   | 22876221     | 59.47              | 96.63   | 54.44  |

Cmls = antennae of Cx. p. molestus; Cqui = antennae of Cx. quinquefasciatus. Alignment rate indicated the ratio of sequencing results to reference genome. Q20 = proportion of base mass values ≥ 20. GC = proportion of GC bases in the sequencing result.

(See figure on next page.)

**Fig. 1** A Principal component analysis of the antennal RNA-Seq data of Cqui and Cmls. B Venn diagram for two sibling species transcripts. C Volcano plot analysis of DEGs between antennal transcriptomes. Green dots indicate DEGs with higher expression in Cqui group, and red dots indicate DEGs with higher expression in Cmls group. D GO enrichment analysis of DEGs more expressed in Cqui group. E GO enrichment analysis of DEGs more expressed in Cmls group.
Fig. 1 (See legend on previous page.)
PPI network for specific functional hub genes

Hub genes were the nodes with higher degree, i.e. nodes with more connections in related pathways. With the cytoHubba plug-in and the Degree algorithm, we calculated the hub gene and drew the PPI Networks, PPI (Fig. 3A). PPI was mainly divided into four parts: CYP family, olfactory-related genes (OR, OBP, and GR) and AchE family and vision-related genes (RBP and Rdh).

The highest scores for specific functional hub genes were CYP4C21 (6044704), OBP lush (6041148), and Rdh12 (6043932), respectively. Hub genes were closely related internally in each specific function. The hub gene score and function are presented in Additional file 1: Table S6.

Among the 30 hub genes, there were ten olfactory-related DEGs, 16 resistance-related DEGs, and four vision-related DEGs (Fig. 3B).

RT-qPCR validation

To validate the reliability of the DEG results, the expression levels of ten selected transcripts were determined by RT-qPCR with the 18S as an internal reference gene. These genes included OBP, Vg, CYP, and so on. All of these genes were significantly different between Cqui and Cmls group on expression of RNA-seq and RT-qPCR (Fig. 4A). The RT-qPCR results were consistent with the transcriptomic data. The correlation coefficient was $R = 0.78$, the significance coefficient $P = 0.0083 < 0.05$ (Fig. 4B).

Discussion

Most previous studies focused on olfactory genes when conducting transcriptome sequencing on mosquito antennae. For example, 77 OBP, 82 ORs, 60 IRs, and 30 GRs were found in the transcriptome sequencing results of different organs of A. albopictus [15]. In the sequencing of the antenna transcriptomes between sibling species An. coluzzii and An. quadriannulatus [35], Cx. quinquefasciatus and Cx. p. molestus [12], much olfactory gene expression was significantly regulated to affect host seeking and oviposition.

In this study, 15 odor-related genes were found among the DEGs between Cx. quinquefasciatus and Cx. p. molestus. Among the olfactory-related DEGs mentioned above, GR22 specifically bound to CO$_2$ [36], and OBP66, general OBP83a, OBP6, general OBP72, OBP12, OBP5, and OBP10 were present specifically in the antennae [37, 38]. Culex pipiens molestus was more interested in mammals and sometimes birds [39, 40]. The blood-sucking host of Cx. quinquefasciatus was more widespread, including birds and other vertebrates [41]. These 15 odor-related genes might reflect the different feeding patterns of the two mosquito species.

**Fig.2** Expression level for specific functional DEGs. Light color-filled columns represent the gene expression of Cqui group; dark color-filled columns represent the gene expression of Cmls group. A Expression level of olfactory-related DEGs. B Expression level of resistance-related DEGs. C Expression level of reproduction-related DEGs. D Expression level of visual-related DEGs.
There were 16 resistance-related DEGs in the hub gene PPI network, including genes from CYP family and AchE. CYP superfamily catalyzed various modification reactions, such as oxidation, epoxidation, dehydrogenation, hydrolysis, and reduction [42]. Members of CYP played critical roles in the detoxification of xenobiotics such drugs, pesticides, and toxins [43]. AchE was an effective insecticide target for mosquito vector control [44]. CYP4AC1 did not overexpress in Cx. quinquefasciatus larvae treated with pyrethroids [45]. AchE1 had been confirmed to be associated with pyrethroid resistance [46]. Higher expression level of CYP6AA9 was found responsible for deltamethrin-resistant Cx. pipiens. [47]. Our study agreed with previous studies confirming the expression levels of resistance-related genes of Cx. quinquefasciatus and Cx. pipiens pallens were different [48]. The relationship between 16 resistance-related DEGs and resistance needs further verification.

The two Vg-A1 genes (6043252,6043250) were highly expressed in Cx. p. molestus, which may be related to the autogenous habit [49]. In the absence of a blood meal, the ovaries of Cx. p. molestus developed normally, which might have led to higher expression of the Vg gene.

Of the eight differentially expressed vision-related genes, RBP1, RXP Rax, eye-specific DGK, and VA opsin were more highly expressed in the Cqui group. Retinal oxide-binding protein was abundantly expressed in locust antenna and associated with olfactory-related behaviors in solitary locusts [50]. Eye-specific DGK was essential for the photoreceptor function of the Drosophila retina [51, 52]. Vertebrate ancient opsin was a green-sensitive photoreceptor that showed high sequence similarity to vertebrate ancient opsin, which might also have affected
sexual maturation [53]. Rdh12 functioned as part of the visual cycle, which was a series of enzymatic reactions required for the regeneration of the visual pigment and detoxification of lipid peroxidation products [54, 55]. Retinol-binding protein transported vitamin A in the hydrophilic environment of the cytoplasm and regenerated visual pigments [56, 57]. RAR was involved in the retinoic acid signaling pathway, which was crucial for the control of embryonic development [58]. The different living environments (Cx. p. molestus mated mainly in enclosed spaces, while Cx. quinquefasciatus mated mainly in open areas) might interact with the differential expression of their vision-related genes.

GO enrichment results showed that genes with higher expression in Cx. quinquefasciatus were mainly enriched in cell communication, G protein-coupled receptor signaling pathway, ion channel complex, cell junction, signaling receptor activity, and other pathways. Previous studies have found that G protein-coupled receptor signaling pathway was closely related to insect feeding behavior [59], which affected insecticide resistance in Cx. quinquefasciatus by regulating P450-mediated detoxification [60].

Genes with higher expression in Cx. p. molestus were mainly enriched in microtubule-based movement, innate immune response, integral and intrinsic component of endoplasmic reticulum membrane synthesis, and cell division-related pathways. Due to the autogenous habit of Cx. p. molestus, the body of Cx. p. molestus was richer in carbohydrates, lipids, proteins, and other nutrients [49, 61, 62], with vigorous cell proliferation and stronger immune response.

The screened high scored hub genes would be the key to explore mechanisms behind the two sibling species biological differences. OBP lush (6041148) had the highest score for olfactory-related hub gene and consisted of a large family of low-molecular-weight, highly divergent proteins expressed exclusively in the chemosensory sensilla of insects. It was required for normal olfactory behavior in Drosophila [63]. OBP lush mutant flies were abnormally attracted to high concentrations of ethanol, propanol, and butanol but had normal chemosensory responses to other odorants [64].

The resistance-related hub gene CYP4C21 (6044704) had the highest score, which was 5.4 times higher in the resistant strain compared with the wild Ae. aegypti strain in Vietnam [65]. The hub visual correlation gene with the highest score was Rdh12 (6043932), an NADPH-dependent retinal reductase, which is expressed in the inner segments of the photoreceptors [54]. Rdh12 could enzymatically reduce toxic lipid 4-hydroxynonenal in vitro [66], protecting cellular macromolecules against oxidative modification and protecting photoreceptors from light-induced apoptosis [67].

Our transcriptome sequencing on antennae of Cx. quinquefasciatus and Cx. p. molestus, not only focused on olfactory-related genes in common view, but also expanded the orientation of resistance-, reproduction-, and vision-related genes of two sibling species of mosquitoes. Even though the RT-qPCR results confirmed the
RNA-Seq prediction to some extent, further molecular and behavioral investigations are needed. This study provides hints about the potential molecular mechanisms behind the two sibling species’ biological differences, like blood-feeding, detoxication, mating, host-locating, and other physiological behaviors, which could facilitate the design and development of more targeted repellents or insecticides. By interfering with or silencing the specific genes with RNAi or CRISPR techniques, mosquito host localization ability and resistance to insecticides were affected, leading to the decrease of vector competence. The screened key genes can help to control mosquito-borne diseases effectively and efficiently.

Abbreviations
DEGs: Differentially expressed genes; PPI: Protein–protein interaction; qRT-PCR: Quantitative real-time PCR; Cqu: Antennae of Culex quinquefasciatus; Cmls: Culex pipiens molestus; NCBI: National Center for Biotechnology Information; GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; BP: Biological process; MF: Molecular function; CC: Cellular components; 18S: 18S ribosomal RNA; OBP: Odorant-binding protein; OR: Odorant receptor; GR: Gustatory receptor; CYP: Cytochrome P450; AChE: Acetylcholinesterase; Vg: Vitellogenin; lov: Location of vulva defective; RAR: Retinoic acid receptor; VA: Vertebrate ancient; DGK: Eye-specific diacylglycerol kinase; RBP: Retinol-binding protein; Rdh: Retinol dehydrogenase; RLBP: Retinaldehyde-binding protein; AchR: Acetylcholine receptor; SCP: Sodium channel protein; EcR: Ecdysone receptor; TPK: Tyrosine-protein kinase.

Supplementary Information
The online version contains supplementary material available at https://doi.org/10.1186/s13071-022-05482-6.

Additional file 1: Table S1. The qPCR primers designed to verify RNA-seq results. Table S2. Olfactory-related differentially expressed genes. Table S3. Resistance-related differentially expressed genes. Table S4. Reproduction-related differentially expressed genes. Table S5. Visual-related differentially expressed genes. Table S6. The hub gene score and function.

Author contributions
TZ and CL conceived, designed the experiments, obtained the funding, and revised the manuscript. QY, XZ, and ZG carried out the experiments (RT-qPCR, total RNA extraction, construction of cDNA library). HG and JL analyzed the sequencing data. HG, ZG, and DX wrote an initial version of the manuscript. All authors read and approved the final manuscript.

Funding
This study was supported by the National Natural Science Foundation of China (grant no. 81371847).

Availability of data and materials
The datasets of this article are included within the manuscript and its supplementary material. All the RNA-seq raw data were submitted to the National Centre for Biotechnology Information (NCBI). Sequence Read Archive with a BioProject ID: PRJNA843865.

Declarations
Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that there are no competing interests.

Received: 22 June 2022 Accepted: 13 September 2022
Published online: 01 October 2022

References
1. Singh M, Suryanshu Kanika, Singh G, Dubey A, Chaitanya RK. Plasmodium's journey through the Anopheles mosquito: a comprehensive review. Biochimie. 2021;181:176–90.
2. Weaver SC, Charlier C, Vasilakis N, Lecuit M, Zika, Chikungunya, and other emerging vector-borne viral diseases. Annu Rev Med. 2016;69:395–408.
3. Lobl M, Thieman TK, Clayer D, Higgins S, Troxbridge RM, Hewlett A, et al. What's eating you? Culex mosquitoes and West Nile virus. Cuts. 2021;107:244–7.
4. Aarden ML, vonHoldt BM, Fritz ML, Davis SR. Global evaluation of taxonomic relationships and admixture within the Culex pipiens complex of mosquitoes. Parasite Vectors. 2020;13.8.
5. Gomes B, Sousa CA, Vicente JL, Pinho L, Calderón J, Arez E, et al. Feeding patterns of molestus and pipsiens forms of Culex pipiens (Diptera: Culicidae) in a region of high hybridization. Parasite Vectors. 2013;6.93.
6. Jaffar-Bandjee M, Steinmann T, Krijnen G, Casas J. Insect pectinate antennae maximize odor capture efficiency at intermediate flight speeds. P Natl Acad Sci USA. 2020;117:281-26.3.
7. Eberl DF. Feeling the vibes: chordotonal mechanisms in insect hearing. Curr Opin Neurobiol. 1999:9.389–93.
8. McMeniman CJ, Corfas RA, Matthews BJ, Ritchie SA, Vosshall LB. Multimodal integration of carbon dioxide and other sensory cues drives mosquito attraction to humans. Cell. 2014;156:1060–71.
9. Wada-Katsumata A, Schal C. Antennal grooming facilitates courtship performance in a group-living insect, the German cockroach Blattella germanica. Sci Rep-UK. 2019.9.2942.
10. Raji JI, DeGennaro M. Genetic analysis of mosquito detection of humans. Curr Opin Insect Sci. 2017;20:34–8.
11. Chen Q, Pei D, Li J, Jing C, Wu W, Man Y. The antenna transcriptome changes in mosquito Anopheles sinensis, pre- and post- blood meal. PLoS ONE. 2017;12.e0183139.
12. Gu ZY, Gao HT, Yang QJ, Ni M, Li MJ, Xing D, et al. Screening of olfactory genes related to blood-feeding behaviors in Culex quinquefasciatus and Culex pipiens molestus by transcriptome analysis. PLoS Neglect Trop D. 2022;16.e0010204.
13. Sanford JL, Shields VN, Dickens JC. Gustatory receptor neuron responds to DEET and other insect repellents in the yellow-fever mosquito Aedes aegypti. Nat Commun. 2013;10:269.73.
14. Carvalho DO, Chuffi S, Ioshino RS, Marques ICS, Fini R, Costa MK, et al. Mosquito pornoscopy: observation and interruption of Aedes aegypti copulation to determine female polyandric event and mixed progeny. PLoS ONE. 2018;13.e0193164.
15. Lombardo F, Salvermini M, Fiorillo C, Nolan T, Zwiebel LJ, Ribeiro JM, et al. Deciphering the olfactory repertoire of the tiger mosquito Aedes albopictus. BMC Genomics. 2017;18.770.
16. Saltin BD, Matsumura Y, Reid A, Windmill JF, Gorb SN, Jackson JC. Material stiffness variation in mosquito antennae. J R Soc Interface. 2019;16:20190049.
17. Dehghan H, Sadraei J, Moosa-Kazemi SH, Baniani NA, Nowruzi F. The molecular and morphological variations of Culex pipiens complex (Diptera: Culicidae) in Iran. J Vector Dis. 2013;30:111–20.
18. Tahiri HM, Kanwäl N, Mehwish. The sequence divergence in cytochrome C oxidase I gene of Culex quinquefasciatus mosquito and its comparison with four other Culex species. Mitochondrial DNA A DNA Mapp Seq Anal. 2016;27:3054–7.
19. Kim D, Paggi JM, Park C, Bennett C, Salzberg SL. Graph-based genome alignment and genotyping with HiSAT2 and HiSAT-genotype. Nat Biotechnol. 2019;37:907–15.
20. Behura SK, Lobo NF, Haas B, deBruyn B, Lovin DD, Shumway MF, et al. Complete sequences of mitochondria genomes of Aedes aegypti and Culex quinquefasciatus and comparative analysis of mitochondrial DNA fragments inserted in the nuclear genomes. Insect Biochem Molec. 2011;41:770–7.

21. O’Leary NA, Wright MW, Brister JR, Ciufo S, Haddad D, McVeigh R, et al. Reference sequence (Refseq) database at NCBI: current status, taxonomic expansion, and functional annotation. Nucleic Acids Res. 2016;44:D733-745.

22. Kovaka S, Zimiv AV, Pertea GM, Razaghi R, Salzberg SL, Pertea M. Transcriptome assembly from long-read RNA-seq alignments with stringTie2. Genome Biol. 2019;20:278.

23. Mistry J, Chuguransky S, Williams L, Qureshi M, Salazar GA, Sonnhammer ELL, et al. Pfam: the protein families database in 2021. Nucleic Acids Res. 2021;49:D41-2-D419.

24. The Gene Ontology resource. enriching a Gold mine. Nucleic Acids Res. 2011;41:770–7.

25. Mi H, Muruganujan A, Ebert D, Huang X, Thomas PD. PANTHER version 14: more genomes, a new PANTHER GO:slim and improvements in enrichment analysis tools. Nucleic Acids Res. 2019;47:D419-d426.

26. Kanehisa M, Sato Y, Kawashima M. KEGG mapping tools for uncovering hidden features in biological data. Protein Sci. 2022;31:47–53.

27. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 2014;15:550.

28. Wu T, Hu E, Xu S, Chen M, Guo P, Dai Z, et al. clusterProfiler 4.0: a universal enrichment tool for interpreting omics data. Innovation. 2021;2:100141.

29. Yu G, Wang LG, Han Y, He QY. clusterProfiler: an R package for comparing biological themes among gene clusters. OMICS. 2012;16:284–7.

30. Szklarczyk D, Gable AL, Nastou KC, Lyon D, Kirsch R, Pyysalo S, et al. The STRING database in 2021: customizable protein-protein networks, and functional characterization of user-uploaded gene/measurement sets. Nucleic Acids Res. 2021;49:D605-d612.

31. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, et al. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res. 2003;13:2498–504.

32. Chin CH, Chen SH, Wu HH, Ho CW, Ko MT, Lin CY. cytoHubba: identifying hub objects and sub-networks from complex interactome. BMC Syst Biol. 2014;8:511.

33. Schmittgen TD, Livak KJ. Analyzing real-time PCR data by the comparative (CT) method. Nat Protoc. 2008;3:1101–8.

34. Bubner B, Gase K, Baldwin IT. Two-fold differences are the detection limit for determining transgene-copy numbers in plants by real-time PCR. BMC Biotechnol. 2004;4:14.

35. Atthey G, Popkin-Hall Z, Cosme LV, Takken W, Slotman MA. Species and comparative analysis of mitochondrial DNA Culex quinquefasciatus, Aedes aegypti, and Aedes taeniorhynchus in Southern House mosquito, Culex pipiens molestus (Diptera: Culicidae). J Med Entomol. 2000;37:726–31.

36. Ma Z, Liu J, Guo X. A retinal-binding protein mediates olfactory attraction in the migratory locusts. Insect Biochem Molec. 2019;114:103214.

37. Mi H, Muruganujan A, Ebert D, Huang X, Thomas PD. PANTHER version 14: more genomes, a new PANTHER GO:slim and improvements in enrichment analysis tools. Nucleic Acids Res. 2019;47:D419-d426.

38. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 2014;15:550.

39. Wu T, Hu E, Xu S, Chen M, Guo P, Dai Z, et al. clusterProfiler 4.0: a universal enrichment tool for interpreting omics data. Innovation. 2021;2:100141.

40. Yu G, Wang LG, Han Y, He QY. clusterProfiler: an R package for comparing biological themes among gene clusters. OMICS. 2012;16:284–7.

41. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 2014;15:550.

42. Wu T, Hu E, Xu S, Chen M, Guo P, Dai Z, et al. clusterProfiler 4.0: a universal enrichment tool for interpreting omics data. Innovation. 2021;2:100141.

43. Yu G, Wang LG, Han Y, He QY. clusterProfiler: an R package for comparing biological themes among gene clusters. OMICS. 2012;16:284–7.

44. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 2014;15:550.

45. Wu T, Hu E, Xu S, Chen M, Guo P, Dai Z, et al. clusterProfiler 4.0: a universal enrichment tool for interpreting omics data. Innovation. 2021;2:100141.

46. Yu G, Wang LG, Han Y, He QY. clusterProfiler: an R package for comparing biological themes among gene clusters. OMICS. 2012;16:284–7.

47. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 2014;15:550.

48. Wu T, Hu E, Xu S, Chen M, Guo P, Dai Z, et al. clusterProfiler 4.0: a universal enrichment tool for interpreting omics data. Innovation. 2021;2:100141.
66. Belyaeva OV, Korkina OV, Stetsenko AV, Kim T, Nelson PS, Kedishvili NY. Biochemical properties of purified human retinol dehydrogenase 12 (RDH12): catalytic efficiency toward retinoids and C9 aldehydes and effects of cellular retinol-binding protein type I (CRBP1) and cellular retinaldehyde-binding protein (CRALBP) on the oxidation and reduction of retinoids. Biochemistry-US. 2005;44:7035–47.

67. Marchette LD, Thompson DA, Kravtsova M, Ngansop TN, Mandal MN, Kasus-Jacobi A. Retinol dehydrogenase 12 detoxifies 4-hydroxynonenal in photoreceptor cells. Free Radical Bio Med. 2010;48:16–25.

Publisher's Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.