The Simuliid Bulletin

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Cover Image: Female of black fly (photo: Samuel Krčmárik)

From the Editor

The most recent issue of the Simuliid Bulletin is dedicated to the genetics of black flies. Charles Brockhouse and his student present another interesting topic from the "Intriguing Genes" series. In this issue, Molly Conway, a student from Creighton University, shares part of her study of Gustatory Receptors in the adult stages of *Simulium vittatum*.

The Simuliid Bulletin welcomes papers from students and encourages black fly specialists and enthusiasts to share their papers on any topics related to black flies that they find interesting or relevant.

Tatiana Kúdelová, Editor

FORTHCOMING MEETINGS

20th Annual North American Black Fly Association (NABFA) Meeting

February 6-9, 2024

Harrisburg, Pennsylvania

The 20th Annual meeting of the North American Black Fly Association will take place along with a 2-day taxonomic workshop focusing on basic black fly taxonomy in Harrisburg, PA. The taxonomic workshop will be led by Mr. Denny Keen of the PADEP, Ms. Carey LaMere of the MMCD and Dr. Peter Adler, Professor Emeriti at Clemson University. The workshop will be held **February 6 & 7** and the NABFA meeting will take place on the **8th & 9th**. Workshop participants are encouraged to participate and present in the NABFA meeting. The workshop will take place at the PADEP laboratory, which has facilities for 25 participants. Workshop participants are encouraged to collect and preserve samples this season to bring to the event.

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The X International Simuliidae Symposium,

September 23-27, 2024

Cappadocia, Turkey

Dear Friends and Colleagues,

We are pleased to announce that the **10th International Simuliidae Symposium** will be held in the richly historic area of Cappadocia, Turkey, in 2024 with tentative dates of **23-27 September.** The symposium will be organized by Erciyes University and Turkish Society for Parasitology. Erciyes University is located in Kayseri, a large modern city of about 1.5 million people, with a convenient airport.

Further information and a call for abstracts will be announced through this mailing list near the beginning of 2024. In the meantime, for any questions, please contact the Chair of the Symposium Organizing Committee, Prof. Dr. Alparslan Yildirim: yildirima@erciyes.edu.tr

We look forward to welcoming the international community of simuliid workers and other interested researchers and public health workers to Turkey in 2024.

Prof. Dr. Alparslan Yildirim
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SCIENTIFIC PAPERS

Note: Using *Simulium* Genomics Data for Undergraduate Teaching

Charles Brockhouse

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No area in science is growing as rapidly as genomics (including allied approaches such as transcriptomics, proteomics, etc.). Virtually every facet of the pure and applied life sciences is being affected by the new sciences. In my advanced Genetics course, Current Topics in Genetics, I am experimenting with teaching genomics in the context of blackflies. Aspects of blackfly biology cover the whole gamut of student interests we find at Creighton University. Pre-health students are fascinated by the disease transmission and the impact of mass biting. Those more interested in ecology can focus on larval filter-feeding which can reveal the metagenomes of riparian systems. Students interested in evolution can focus on sibling species, and intraspecific variation.

A significant part of the course consists of students using the JAMp transcriptome annotation system that we have been presenting in the Simuliid Bulletin in the "Intriguing Genes" series. Students are assigned or choose a particular Gene Ontology term (GO term: www.geneontology.org), retrieve *S. vittatum* and *S. tribulatum* sequences that are so annotated, and examine the expression levels at different developmental stages and protein structure, and infer possible biological functions of the gene(s) in the context of blackfly biology. In the process, the students learn to use biological databanks such as UniProt and NCBI, basic skills such as BLAST searches and sequence alignment, and genome sequence assembly. And of course, they are immersed in the biology of the most fascinating family of organisms.

All of the sequences presented in the Intriguing Genes series are now deposited in a NCBI Bioproject multispecies sequencing study (Accession: PRJNA911831, ID: 911831). In this issue, Molly Conway, a student from the spring of 2023 presents part of her study of Gustatory Receptors in the adult stages of *Simulium vittatum*.

Intriguing Genes: Expressed Sequences from the *Simulium vittatum-tribulatum* complex. IV. Sex Differential Expression of Gustatory Receptors in *Simulium vittatum*.

Molly Conway

Current Topics in Genetics Bio 517, Biology Department, Creighton University, Omaha, NE, 68178 USA

Abstract:

Developmentally stage annotated transcriptomes of *Simulium vittatum* and *S. tribulatum*, were searched with the expression "gustatory receptor" retrieving 12 expressed sequences. Four of these showed differential expression in the adults' stages between males, newly emerged females (nulliparous) and 3-day post-egg laying females (parous). By combining the known functions of orthologues in other organisms with the expression patterns observed in S. vittatum, it is possible to infer the role of these gustatory receptors in mate choice, sugar meal acquisition and possibly ovipositioning.

Introduction

Insect gustatory receptors fall into the insect family of large G-protein coupled receptors. While the insect gustatory receptors class is fully independent functionally and morphologically, they are distantly related to insect olfactory receptors as both classes are chemoreceptors (Isono & Morita, 2010). The primary link between gustatory and olfactory receptors is their common amino residue motif located in the seventh transmembrane plus C terminal domain, indicating that they evolved from the same ancestral chemoreceptor family (Isono & Morita, 2010). The insect taste receptor system works to detect pheromones and somatosensory stimulants and is present in a wide spectrum of ligand sugars, bitter substances, and salts that are routinely found in mammals (Morinaga, et. al., 2022). Researchers are still working to determine the precise central mechanism for insects perceiving and differentiating taste information, as it is not yet elucidated (Isono & Morita, 2010).

Insect taste organs are simple hairlike structures full of taste neurons present on the distal legs of the fly working to promote the feeding reflex reaction when exposed to sugar stimulations in Drosophila melanogaster, (Isono & Morita, 2010). The schematic structure of the gustatory organs responsible for taste allows for single-unit action potentials, reactivity to taste ligands and other physiological mechanisms, (Isono & Morita, 2010). Insect taste organs are stimulated by a sugar solution leading to the induction of eating behaviours, proboscis extension response, and a variety of neuronal gustatory responses (Isono & Morita, 2010).
D. melanogaster possesses taste receptors on the mouth parts, but also have gustatory receptor neurons throughout their legs and wings due to their tendency to walk along the surface of their food before consuming it (Yarmolinsky, et. al., 2009). When *D. melanogaster* ingest food it enters through a muscular tube, the proboscis, then pushes past the two labial palps gating the pharynx (Yarmolinsky, et. al.). *D. melanogaster* have approximately 200-300 gustatory receptor neurons distributed on the proboscis, legs, and wings (Yarmolinsky, et. al., 2009). Gustatory receptor neurons line the pharynx and internal taste organs, both labial palps contain 31 taste bristles on their outer surfaces and there are 30 additional taste pegs exposed between the furrows of pseudotrachea when the fly engages in feeding activities (Yarmolinsky, et. al., 2009). All gustatory neurons in *D. melanogaster* connect a dendrite to a terminal pore at the furthest end of the bristle shaft and the axonal process typically terminates in the subesophageal ganglion (Yarmolinsky, et. al., 2009).

Gustatory receptors were selected for the gene ontology term due to interest in the mechanisms of these receptors and learning about how they differ from the other chemoreceptors and senses present in *Simulium tribulatum* and *S. vittatum*. Taste reception and perception is intellectually intriguing through how similar reception mechanisms can yield a wide variety of taste perceptions, such as sweet and bitter. Gustatory receptors are biologically significant as research to learn more about the different gene sequences and expression patterns of Simuliidae gustatory receptors could tell researchers more about blackfly taste receptors and what they are attracted to. Learning more about the biological significance of their taste receptors could ultimately help discover new mechanisms to help repel insects from humans and other mammals potentially preventing some insect-borne diseases.

Materials and Methods:

This study was conducted as previously described for the "Intriguing Genes" series (Brockhouse et al., 2020). For this paper, the annotated transcriptomes were searched with the expression "gustatory receptor". Four of the twelve retrieved sequences, that showed differential expression among developmental stages, are presented here. Uniprot (www.uniprot.org) searches were conducted automatically by the JAMp system previously described to find functionally-verified orthologues.

Results and Discussion:

c185367_g1_i1|m.74526

The expression figure of c185367_g1_i1|m.74526 (Figure 1) shows that this gene is expressed in male *S. vittatum* and in parous females. Uniprot hits (www.uniprot.org; see Table 1) suggest its function to be one of the few identified sugar gustatory receptors that have been identified so far that promotes the starvation-induced increase of feeding motivation. It is required to be combined with Gr64f to detect sucrose, maltose, and glucose. Its stated molecular function is a sweet taste receptor activity as well as general taste receptor activity, and the stated biological function is detection of chemical stimulus involved in sensory perception of taste and detection of glucose, maltose stimulus, and sucrose stimulus. The subcellular location is a multi-pass membrane protein in the cell membrane that has features of topological domains and transmembrane domains. Tissue specificity has been identified as expression in Gr5a-expressing sugar sensing cells and its induction is linked to expression that is increased by starvation.
The expression in both males and females suggest that in blackflies

this receptor might be used to secure sugar meals for flight energy. *S. vittatum* is a primaparously autogenous species, not requiring a blood meal for the first egg batch. However, subsequent rounds of oogenesis do require a blood meal, and so post-egg laying females may prepare to detect sugar meals for flight energy. Similarly, males may require sugar meals for energy to fly in mating swarms.

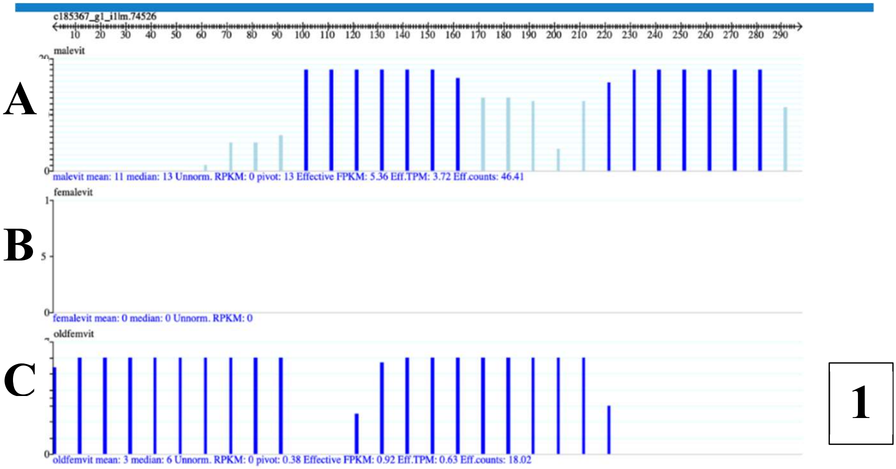

Fig.1. Expression levels of gustatory receptor c185367_g1_i1|m.74526 in adult *S. vittatum*. A: Males. B: Nulliparous females. C: Parous females. The horizontal axis represents the length of the expressed sequence. The vertical bars show the relative number of times that spot was sequenced from the stage-specific mRNA, thus the relative expression levels. This gene, which contributes to sugar detection and feeding behaviour in Drosophila, is expressed in both males and parous females.

c127215_g1_i1|m.6268

The expression profile of c127215_g1_i1|m.6268 **(**Figure 2) shows that the gene is expressed in male *S. vittatum.* The protein sequence matches in Uniprot (Table 1) suggest the function of this gene as a chemoreceptor that mediates acceptance or avoidance behaviours depending on what substrate is attached to. In *Drosophila*, it functions as a receptor and transducer. Its subcellular location was identified as the cell membrane, as it is a topological domain and transmembrane protein. It is involved in both repulsion and attraction behaviour, depending on the substate it is bound to. Expression of some portions of the gene were also noted in *S. tribulatum* larvae (data not shown), potentially indicating stage-specific alternative splicing of the mRNA to yield functionally different gustatory receptors.

The presence only in male adults raises the possibility that this protein is involved in mate recognition, after contact following mating swarms.

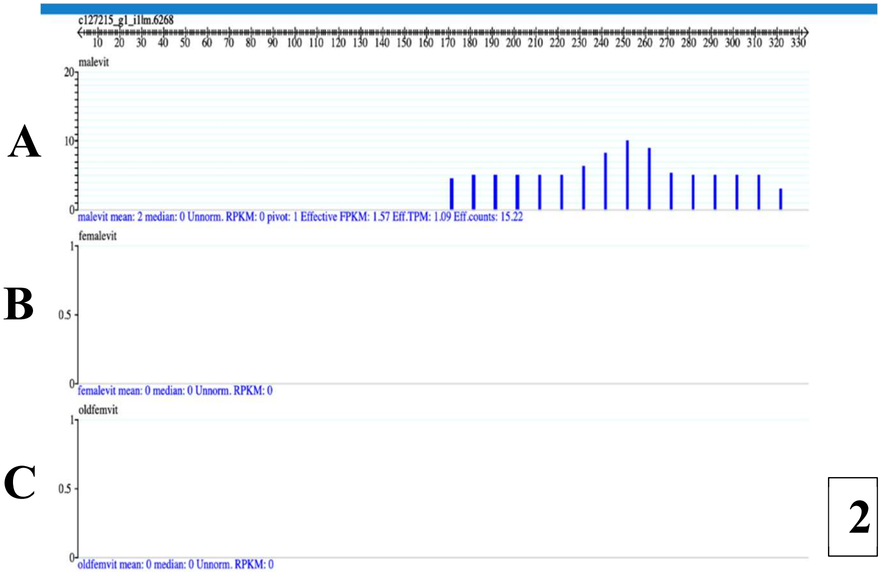

Fig.2. Expression levels of gustatory receptor c127215_g1_i1|m.6268 in adult *S. vittatum*. A: Males. B: Nulliparous females. C: Parous females. The horizontal axis represents the length of the expressed sequence. The vertical bars show the relative number of times that spot was sequenced from the stage-specific mRNA, thus the relative expression levels. Expression of this genes is restricted to males in the adults.

c153842_g1_i2|m.25574

The expression figure of c153842_g1_i2|m.25574 (**Figure 3**) shows that this gene is expressed only in newly emerged/nulliparous female *S. vittatum* adults. The Uniprot matches (Table 1) indicate the gene's function as a probable gustatory receptor which mediates acceptance or avoidance behaviour which is dependent on what substrate it is with. In multiple species, the stated molecular function is a G protein coupled receptor and taste receptor activity, while the biological process is detection of chemical stimulus involved in sensory perception of taste. Its molecular function is a receptor and a transducer, it serves as a putative gustatory receptor. As for subcellular location in *Drosphila*, it is in the cell membrane and has features that align with topological domains and transmembrane proteins. Tissue specificity features show that it is expressed in neurons of the terminal chemosensory organ, the dorsal chemosensory organ, and dorsal pharyngeal sense organ of the larvae.

The expression in nulliparous but not parous females suggest that it could be involved in mate recognition or the selection of oviposition sites (e.g., detection of the aggregation contact pheromone proposed by McCall, 1995 and McGaha et al., 2015).

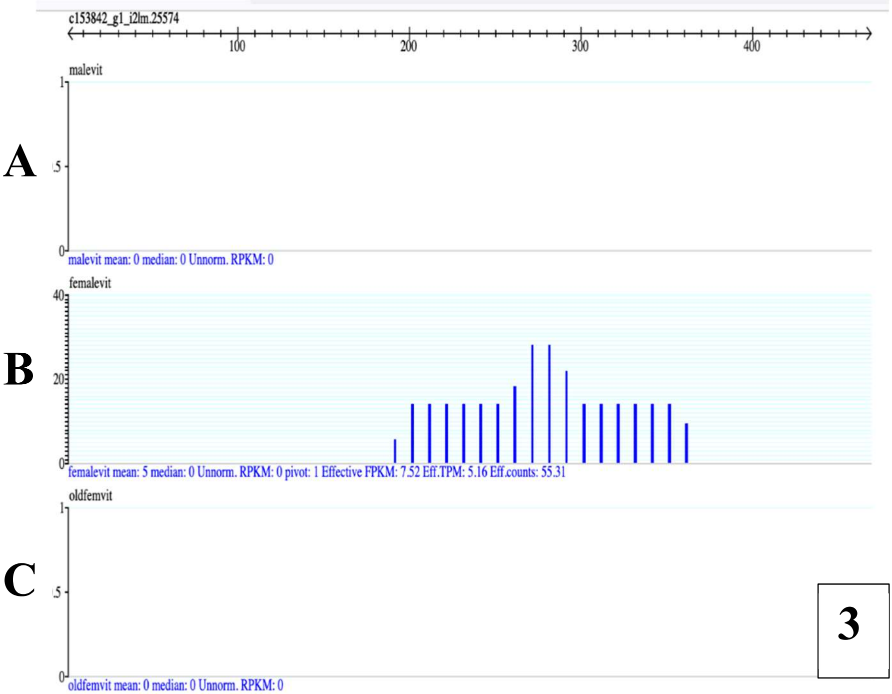

Fig.3. Expression levels of gustatory receptor c153842_g1_i2|m.25574 in adult *S. vittatum*. A: Males. B: Nulliparous females. C: Parous females. The horizontal axis represents the length of the expressed sequence. The vertical bars show the relative number of times that spot was sequenced from the stage-specific mRNA, thus the relative expression levels. This gene has detectable expression only in nulliparous females.

c120728_g1_i1|m.5402 -
This is a second nulliparous/newly emerged adult female-specific gustatory receptor (Figure 4). The protein matches in Uniprot are involved in avoidance or acceptance behaviour depending on the substrate. As with the previous sequence, c120728_g1_i1|m.5402 could well be implicated in either mate choice or ovipositioning behaviour.

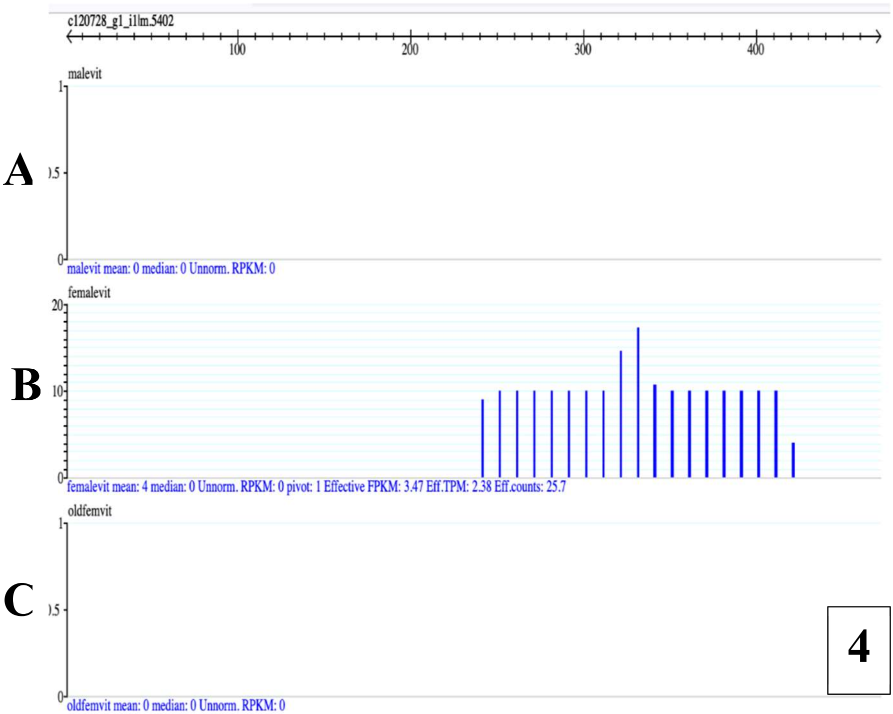

Fig. 4. Expression levels of gustatory receptor c120728_g1_i1|m.5402 in adult *S. vittatum*. A: Males. B: Nulliparous females. C: Parous females. The horizontal axis represents the length of the expressed sequence. The vertical bars show the relative number of times that spot was sequenced from the stage-specific mRNA, thus the relative expression levels. Expression of this genes is restricted to newly emerged (nulliparous) females.

Conclusion:

Gustatory receptors, and the related odorant receptors, are important components of simuliid biology that have the potential as a basis for new control strategies. Sugar feeding, mate acquisition and oviposition are all functions that could have a major impact on pest populations when interrupted. The colony *S. vittatum* sequences given here can be used to retrieve the homologous sequences for important pest and vector species that may be less amenable to developmental staging.

Table 1.

Sequence Name	Protein Sequence	UniProt Hit ID	*S. vittatum* Expression
c185367_g1_i1|m.74526	SSYLFFVVLASKW KPIMC MWREKELVFLKKP YTIKRL PLNRTINVVAFGI IFFAGVV EHLFYSAQSFYAK MTDAAHCNIT VPHALNYYFQYEF SNIYQT	**P83293 · GR64A_DROME**	**Adult males: FPKM 5.36** Parous females: FPKM 0.92
c127215_g1_i1|m.6268	LGFQLTMSFGQNA STMIYVTFVVAV TSQLYIYAQGGQY LINESQLVSNAI YSSVWYEAEPKVR KKLVLALMRAQK AVTVDGVFFEAAL PS FTSILSTAASYTT FLQSIDK*	**P81921 · OR47A_DROME Q9W2U9 · OR9A_DROME 9VZW8 · OR63A_DROME**	**Adult males: FPKM 1.57**
c153842_g1_i2|m.25574	KEFDNCAQKVVQT TTFDEMLVIRG LLNRMFDLSSLIN RLFGWSMLVNV GNDFVAITSNSYF VFVYFQDTTIQ NKDSWRILGSLIW SAPHLFNIVLL TAVCHSTVETMEH LSLQLLHQKVA FTARGFFNVDFTL LFTMIGATTTY LIILIQFHMSE*	**Q9W594 · GR02A_DROME**	**Nulliparous females:** FPKM 7.52

c120728_g1_i 1|m.5402	HFWIPFVGWGVAV DVAVYKTMWGSF QVRYFRVTGESLK FPNLKILIVLFS IGCLVCAILFLIS LSLLLEGFSLWH TTAYYHIITMLNM NSALWYINSRGI RVASNSLAKCFRK DVGIECTAALIS QYRFLWLNLSEML QALGNAYARTYST YCLFM*	**Q7PMG3 ·GR22_ ANOGA** **Q9VPT1 · GR21A_DROME**	Nulliparous female: FPKM 3.47

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Notes for Contributors

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Content covers scientific papers, short research notes, notices and accounts of meetings, and articles of anecdotal or general interest that would not normally be found in international journals. Geographical cover is world-wide. Reports of research carried out by graduates, young scientists and newcomers to the subject are particularly encouraged. It is an ideal medium for offering new ideas and stimulating discussion because of the very short interval between acceptance and publication. Contributions may be accepted up to two weeks before the publication dates at the end of January or July.

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