The Simuliid Bulletin

Number 56 July 2021

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Cover Image: *Simulium thyolense*, a *S. damnosum* sibling species from Malawi (supplied by Daniel Boakye)

From the Editor

The meeting section of the current issue of the Simuliid Bulletin is short. Because of the covid-19 pandemics and travel restrictions, we had to cancel the IX International Simuliidae Symposium again. The international blackfly conference is planned for 2022, and the same situation is with the NAFBA meeting.

However, after a long time of social distancing, there is again the opportunity to meet face-to-face at the Xth International EMCA Conference in Vienna. You might be interested in the conference section dedicated to Blackflies in Europe.

Stay safe and healthy

Tatiana Kúdelová, Editor

FORTHCOMING MEETINGS

10th EMCA Conference: "New insights into mosquito and blackfly control"

The President and the EMCA Board together with the organising committee led by Hans Jerrentrup, cordially invite you to attend our 10th International EMCA Conference to be held at AGES in

Vienna, Austria,

from 3rd to 7th October, 2021.

We are very pleased to invite you to the **in-person International EMCA Conference** in the beautiful city of Vienna. Since travel restrictions due to the Covid-19 pandemic have been eased (given that travelers are in possession of a vaccination certificate or a negative Sars-Cov-19 virus PCR test), it is a good opportunity to meet again face-to-face. The city of Vienna, situated in the middle of Europe, will offer an excellent start to resume our lively and inspiring meetings.

1. General information
The participants will arrive on Sunday, 3rd October, and enjoy an evening welcome cocktail.
The conference will start officially on Monday 4th October morning and last through Thursday afternoon 7th October, with a succession of scientific sessions (oral and posters), round tables, and social events.

2. Travel
Vienna is reached conveniently by air, train and car. Easy and rapid connections between the airport and the city of Vienna are available. Check Vienna Public Transport for details.

3. Accomodation
Hotels with different price categories are available for self-booking. We have arranged special conditions for EMCA 2021 participants. The room contingents (double room, single use) are available until

a mid-September at the following hotels:
Hotel Breitenlee (59 EUR per night)
Hotel Accor Ibis Wien Messe (82 EUR)
Hotel Arcotel Donauzentrum (99 EUR)
Hotel Novotel Suites Wien City Donau (109 EUR)
Hotel Arcotel Kaiserwasser (109 EUR)

4. Registration fees

Registration fees include access to all sessions, breaks, lunches, and social events (except IAEA visit). The invoice will be sent to you as of September.

Regular and sustaining members: 390 €

Non-members: 490 €

Student member: 200 €

Student non-member: 250 €

Accompanying person: 130 € (incl. are all breaks and social events)

Optional visit of the International Atomic Energy Agency facilities at Seibersdorf, with a limited number of participants on pre-registration only (due to security-check requirement): 15 €

However, it remains the personal responsibility of the participant to inform himself/herself about all rules and precautionary measures of his home country and the hosting country for traveling, and to follow them. In addition, EMCA advises to take a travel insurance. The booking of suitable accommodation is up to the participant.

5. Timetable for the planning of the conference

Beginning of registration and submission of abstracts: **June 14th**.

End of abstract submission[1]: **August 15th**.

Late registration without abstracts (including on place): after **August 15th**

End of registration: **September 15th**

[1] Abstracts will only be considered for acceptance when the author's registration fee is paid.

6. Scientific topics

There will be room for eight sessions. Some will be introduced by keynotes. The scientific committee has preselected the following session themes:

- Biocides regulation and advancement in insecticides development
- Biocides testing in perspective of registration
- Blackflies in Europe: where are we, where do we go?
- Citizen science and community involvement for mosquito surveillance and control
- Control of vector and harmful insects: improvement of methods and quality assessment
- Decision making processes in mosquito control
- Latest challenges and responses in mosquito control
- Mosquito control without borders in the Danube region
- Mosquito control in urban context
- Mosquito control versus nature conservation: opposition or partnership?
- New technologies and practices in surveillance and control
- Surveillance and management of invasive species
- Surveillance of vector-borne pathogens in insects

6 students awards will be given for best oral and poster presentations of total **1.200euros!**

7. Excursions and conference dinner

The excursion will bring us on Wednesday afternoon to March/Morava flood plains including live demonstrations of mosquito control generously offered by the "Verein biologische Gelsenregulierung". It will be followed by a visit to Castle Schlosshof where a Heurigen dinner will be offered.

As an option, a visit is proposed on Thursday afternoon to the site of the International Atomic Energy Agency (IAEA) in Seibersdorf (SIT insect rearing facilities). The number of participants is limited to 60 persons due to security reasons (first registered, first served).

You can find more details at the confernece website:

https://akademie.ages.at/10th_emca_conference_new_insights_into_mosquito_and_blackfly_control/

WORLD BLACKFLIES (DIPTERA: SIMULIIDAE): A COMPREHENSIVE REVISION OF THE TAXONOMIC AND GEOGRAPHICAL INVENTORY [2021]

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The new blackfly inventory is available at the website:

https://biomia.sites.clemson.edu/pdfs/blackflyinventory.pdf

The present revision of the Inventory of the world's Simuliidae continues the intent to provide yearly, fully updated electronic revisions of the World Inventory, which originally was issued in paper format by Crosskey (1988). The current revision, thus, includes all information known to have been published before 1 January 2021. The purpose and format of this inventory remain the same as for previous revisions.

In this most recent revision of the Inventory, 2,401 species (2,384 living and 17 fossil) are listed as valid), representing a net increase of 53 living species since the previous [2020] revision.

SCIENTIFIC PAPERS

Intriguing Genes: Expressed Sequences from the *Simulium vittatum-tribulatum* complex. III. Flight Behaviour Related Genes (GO: 7629).

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Introduction

We continue our preview of the full *S. vittatum-tribulatum* complex transcriptome, highlighting genes we feel are of interest to the blackfly community, with this release of expressed sequences annotated with the Gene Ontology "Flight Behaviour" (#7629). A total of 146 sequences are annotated with the 7629 GO term in the five libraries (*S. vittatum*: adult males, pre-oviposition adult females, and post-oviposition adult females. *S. tribulatum*: mixed stage female larvae, and mixed stage male larvae). Here we present 17 representative sequences showing a variety of developmental expression patterns. To iterate, most of these sequences have multiple GO terms, and so their expression patterns may reflect other functions than flight behaviour (Figure 1 A & B).

One might reasonably expect that the expression of flight behaviour genes would differ among adult males, newly emerged females and post-ovipositioning females. Adult males must form mating swarms and look for sugar meals, newly emerged females will fly to search for mates, in most species search for blood meals, and oviposit, while parous females will switch (back) to prey searching.

S. vittatum and *S. tribulatum* are both primaparously autogenous: adult females can produce an egg batch without a blood meal, given sufficient feeding as larvae. After the first egg-batch, females will often seek blood meals for a second gonotrophic cycle. The *S. vittatum* colony at University of Georgia (Athens) has been maintained for many years as a completely autogenous population, with heavy selection for altered mating and oviposition behaviour (Gray and Noblet, 1999), so it is possible that the colony's expression of flight behaviour related genes is significantly different from natural populations of *S. vittatum*. Nevertheless, many of the sequences retrieved with this GO term show very different expression levels among males, pre-oviposition, and post-oviposition females. Example profiles are given for several of the differentially expressed genes.

The bulk of the sequences fell into 3 main categories: G protein-related, protein kinases, and neuron growth control/development. The first two categories are involved in signal transduction (relaying information from "the outside" to the cell), while the last could also clearly be involved in setting different behaviours.

Results

Figure 1 depicts the number of sequences with additional GO annotations, in terms of Biological Process (1A) and Molecular Function (1B). Most genes are pleiotropic, involved in several processes. Often, it is easy to see how the processes are related to one another. Flight behaviour certainly involves "Synanaptic Transmission" and kinase activity, for example. Less obviously related multiple annotations might reveal unexpected relationships among processes, or could simply reflect true pleiotropy, where the gene has multiple functions.

In the following section, we present the cDNA and inferred protein sequences of 17 exemplar sequences, with expression level information for the 5 developmental stages of *S. vittatum* and *tribulatum* represented by the libraries. The expression levels are given as "Effective Counts", that is the number of times reads mapping to the gene were sequenced standardized to the relative size of each library. Higher effective counts reflect greater expression at a particular stage.

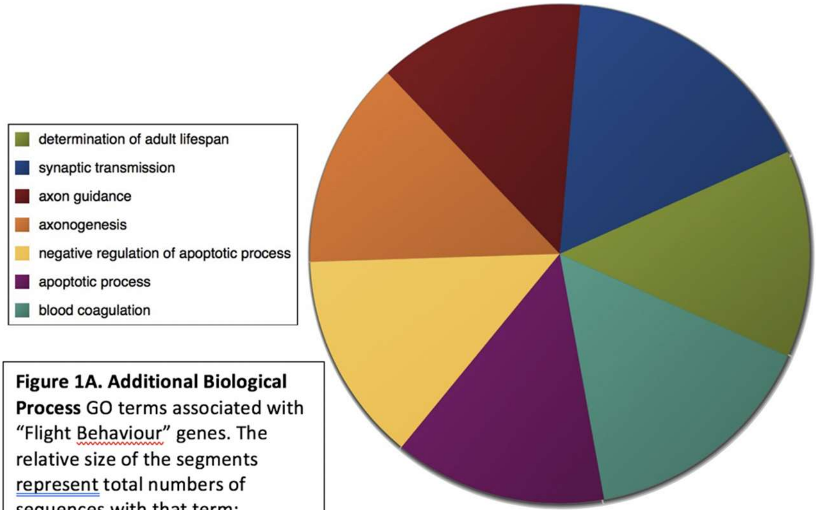

Figure 1A. Additional Biological Process GO terms associated with "Flight Behaviour" genes. The relative size of the segments represent total numbers of sequences with that term; sequences can have more than one GO term attached.

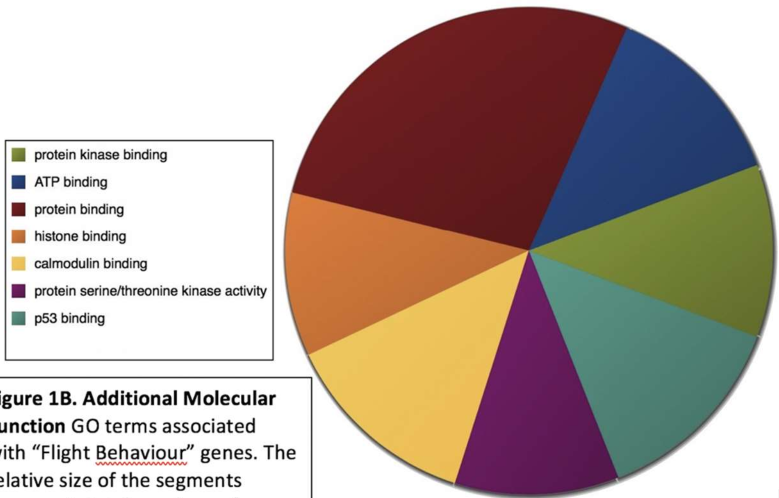

Figure 1B. Additional Molecular Function GO terms associated with "Flight Behaviour" genes. The relative size of the segments represents total numbers of sequences with that term; sequences can have more than one GO term attached.

c151893_g2_i5|m.21230
This sequence is highly expressed in adult male *vittatum* and adult post-oviposition female *vittatum*. Its protein matches largely consisted of cyclin-dependent kinases, as well as cell division regulation proteins.

Effective counts:
Adult male *vittatum*: 321
Adult nulliparous female *vittatum*: 90.45
Adult post-oviposition female *vittatum*: 307.01
Female *tribulatum* larvae: 33.46
Male *tribulatum* larvae: 33.54

>c151893_g2_i5|m.21230
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AATCGTGTACAAATGTCGTGACCGTGACACCGGAAATCTAGTGGCCCTCAAACG
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AAACCTGGTTTGCCTCCTCGAAGTG
TTTAGAAGGAAAAAACGCCTACACCTCGTGTTCGAGTTTTGTGAGCACACCGTC
CTGCATGAATTAGAGCGGAATCCACA
AGGCTGTCCAGACAATCTAACCAAACAAATCACATACCAGACGCTCCTCGGTGT
GGCCTACTGTCACAAACAAGGATGCG
TGCACCGGGACATCAAGCCTGAGAATATACTTTTGACGGCACAGGGGCAGGTC
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>c151893_g2_i5|m.21230
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EVFRRKKRLHLVFEFCEHTVLHELERNPQ
GCPDNLTKQITYQTLLGVAYCHKQGCVHRDIKPENILLTAQGQVKLCDF
GFARMLSPGENYTDYVATRWYRAPELLVGDT
QYNAAVDVWAIGCVFAELIRGDALWPGRSDVDQLYLIRRTLGDLLPRHL
QIFNQNDFFKNITLPVPPNLEPLETKLPSRT
VSNFQMIDFLKKCLDKDPARRWTSERLTTHPVFSDYVAQDKELEMTGTV
SSSASATLQNTNNGVSAYHPKQALLLNRDNK
NKFSNTSLPQLPGQVEIRMPLRNAYPRSDHHLPTI*

c162198_g1_i2|m.58923

Expression of this sequence is much higher in the adult post-oviposition female group than any other; by its Uniprot matches, it appears to code for an ATPase, possibly calcium-transporting.

Effective counts:
Adult male *vittatum*: 469.96
Adult nulliparous female *vittatum*: 144.84
Adult post-oviposition female *vittatum*: 1112.87
Female *tribulatum* larvae: 266.47
Male *tribulatum* larvae: 196.85

>c162198_g1_i2|m.58923
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CTACGGGTGGACATCCGGACAGGCCTGC
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GIIILGTLWVFQREMADGSGGKTKRDTTMTFTCFVLFDMWNALSCRSQTKSIFSI
GFFSNKMFLFAVGFSLIGQLAVIYF
PPLQMVFQTEALSGMDILFLVALTSTVFWVAELKKAFERAMERRVYRKQHVDLDF
V*

c158095_g2_i4|m.39273

This sequence had high expression across the board, with the highest number of counts being in adult male *vittatum*. Uniprot database matches indicate it codes for either an alpha-actinin or a spectrin beta chain. Gene Ontology annotations suggest its function lies in cytoskeletal bundling.

Effective counts:
Adult male *vittatum*: 15233.09
Adult nulliparous female *vittatum*: 5142.86
Adult post-oviposition female *vittatum*: 9494.62
Female *tribulatum* larvae: 14439.57
Male *tribulatum* larvae: 10926.99

>c158095_g2_i4|m.39273
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CGTTGGAGGATCAGTTGCACCGTTTGCG
CGAGTACGAGCAGGCGGTGTACGCGTACAAGCCGCACATTGAGGAGCTGGAG
AAGATCCACCAGGCCGTGCAGGAGTCGA
TGATCTTCGAGAATCGGTACACACAGTACACGATGGAGACGTTGCGTGTGGGA
TGGGAACAGTTGCTCACGTCGATCAAC
AGGAATATCAATGAGGTTGAAAACCAAATCCTGACCCGCGACTCTAAGGGCAT
CACACAAGAGCAACTGACCGAATTCCG
GGCCAGTTTCAATCACTTCGACAAAAATCGCATTGGCCGTCTGACCCCCGAAG
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GCTACTCGATCGGCAAGGACCGTCAAGGTGAAATGGACTTCCAACGCATCATC
GCCGTGGTCGATCCCAACTCAACCGGT
TACGTACAGTTTGACGCCTTCTTGGACTTTATGACGCGCGAAAACACCGACAC
CGACACAGCCGAACAGGTGATCGACTC
GTTCAGGATCTTGGCTTCGGATAAGCCCTACATACTGCCAGACGAACTCCGCC
GCGAATTGCCACCAGACCAAGCCGAAT
ACTGTATCCAACGCATGCCACCATTCAAGGGACCCGGCGCTGCACCCGGCGC
CCTCGACTATATGTCATTCAGTACCGCG
CTGTACGGCGAAAGTGATTTGTAAATTTAAATAACACAAATTGAATAGTGTCTC
GAAACTAATTTACTATTTATTTAAAA
AAACAAACAAAGAAAGAATGTGTTGGAAAACCGAATTATATGGGACTCAAGAT
CAAAGTTACAAAAACGTTCGAAAAGAA
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CGTTTTTTGTCGATTTTTAGTTAA
TTTTCTTTTGTTTTGACATTTTAAACAAATATAAAAAATGATATCCGTCAAAATG
ACAGCTTGAAAAAAGAAGAAACAAG
AAACAAGATGGCAGCATACGAGTTGGGTGGGAAACGAGAGGCACGCACGCGC
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TTTATTTTCTAATTTAACTGAAAA

>c158095_g2_i4|m.39273
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KPDRGKMRFHKIANVNKALDYIASKGVKLVSIGAEEIVDGNLKMTLGMIWTIILRF
AIQDISVEEMTAKEGLLLWCQRKT
APYKNVNVQNFHLSFKDGLAFCALIHRHRPDLIDYSKLSKDNPLENLNTAFDVAE
KYLDIPRMLDPDDLINTPKPDERAI
MTYVSCYYHAFQGAQQVGYLIPLDKHFADRNAETAANRICKVLKVNQENERLMEE
YERLASDLLEWIRRTMPWLASRQSD
STLAGVQKKLEEYRTYRRKHKPPRVEQKAKLETNFNTLQTKLRLSNRPAYMPTEG
KTVADITNAWKGLEGAEKSFEEWLL
AETMRLERIEHLAQKFKHKADTHEDWTRGKEEMLQSQDYKNCRLYELKALKKKH
EAFESDLAAHQDRVEQIAAIAQELNT
LEYHDCVSVNARCQRICDQWDRLGALTQRRRQALDDMERILEKIDILHLEFAKRA
APFNNWLDGAREDLVDMFIVHTMEE
IQGLMSAHDQFKATLGEADKEFNVIVGLVREVESITNQHQIAGGLENPYTTLTAN
DLTRKWSDVRQLVPQRDQTLTNELR
KQQNNESLRRQFAEKSNAVGPWIERQMDAVRAIGMGMTGSLEDQLHRLREYEQ
AVYAYKPHIEELEKIHQAVQESMIFEN
RYTQYTMETLRVGWEQLLTSINRNINEVENQILTRDSKGITQEQLTEFRASFNHFD
KNRIGRLTPEEFKSCLVSLGYSIG
KDRQGEMDFQRIIAVVDPNSTGYVQFDAFLDFMTRENTDTDTAEQVIDSFRILAS
DKPYILPDELRRELPPDQAEYCIQR
MPPFKGPGAAPGALDYMSFSTALYGESDL*

c161260_g6_i2|m.53629

Interestingly, this sequence is not expressed at all in adult nulliparous female *vittatum* but is expressed quite highly in adult parous female *vittatum*. Its protein matches are kinases.

Effective counts:
Adult male *vittatum*: 5.8
Adult nulliparous female *vittatum*: 0
Adult post-oviposition female *vittatum*: 115.51
Female *tribulatum* larvae: 7.25
Male *tribulatum* larvae: 6.39

>c161260_g6_i2|m.53629
ATCTTAGGAAATCTTGCACTTAATCAAGACCAATTTTCTCGCTCAGATATAATG
ATTCCCACCTCCCTGCTTCCAGGAAT
GCAATACTTGGAGTCTCAGCACTTTGTGCATCGCGATCTGGCCGCTCGCAACA
TCCTCCTCGCCTCCCGCAACCAAGCGA

AAATCTCCGATTTCGGACTCTCACGCGCCCTCTGCGTCGGCAACAACTACTAC
CAGGCGTCACAGGGCGGTAAGTGGCCC
ATCAAATGGTACGCTCCCGAATCCTTCAACTTTGGCACCTTTTCGCACGCATCG
GACGTTTGGAGCTTTGGCGTCGTACT
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>c161260_g6_i2|m.53629
ILGNLALNQDQFSRSDIMIPTSLLPGMQYLESQHFVHRDLAARNILLASR
NQAKISDFGLSRALCVGNNYYQASQGGKWP
IKWYAPESFNFGTFSHASDVWSFGVVLWEMFSLGLPPFGDLK

c157132_g1_i1|m.35709

Protein matches indicate this sequence codes for a semaphorin, and it is most highly expressed in adult male *vittatum*. Gene Ontology annotations suggest a role in neuronal development.

Effective counts:
Adult male *vittatum*: 283.9
Adult nulliparous female *vittatum*: 27.37
Adult post-oviposition female *vittatum*: 41.5
Female *tribulatum* larvae: 40.24
Male *tribulatum* larvae: 70.96

>c157132_g1_i1|m.35709
CGCAGATCTGGTCAATGACTTGAATTTTCAGCATTGACGCCGTTAATTTCACCA
ACGCACGGGGTGGTGTGCTTGCGAAA
ATTAGCTATACTTGTACGCGGCGCCTCCACGTTATCCTAAATTCTACGCCAGTG
GTGACAAAACCTTCATTTTTAGTCCA
TACTCGTCTGTGTTGATTCAATCAAAACCTCAAAATATAGGAAATTTCTTAGTTT
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ACAATTCAAAAACTCGTTAGTGGACGATCGTATAGTTCTATCGGTAGTGTTGTA
GTGAAAAAGTGAACTTTCAATCATCA
TGTTGATGTAGTCTGAGTCAATATTGGCCAATCCGAGCATCAAATTATTACTAA
CTGTTGAACAAGCTATCGAAAGAATT
GATTGAGCCAATAAAAAAAGAAGCAAAAAAAAATCGATTGTGAAGTTTGTGTG
TTTTTGGTACTCCGGCAGTGACAGAGC
TGAAAACCACGCCTGAATCAGTGAATTTTGATTGCGGATTGCAAACAACAAATA
ATAGTTGGGAGTTTTTTTCATTGCCG
ACACTATGCTGCTGTTTGTGGAAGTTTTAACAGCATTTATGCTTCTAAGTGTTA
ACATTCAATCAATAGAATGCATGACA
GAACAATTGTCGCCTGATCATGTCCGTGAATTTAGTTGCGGCAAATTCTACAAT
CGTCTGTTTTATTTGGACGAGGAACG
TGACAGCCTCTATGTGGGCTCGATGGATCGTGTTTTCAAGTTGAACCTAGAGA
ACATAAGCACAGCTGCATGTGATCGTG

ACCAAATCCTTCTGGAACCCACTGGCTCTGACGTTGTCAACTGTGTTTCCAAGG
GCAAGTCTCAGCTCTTCGATTGTCGC
AACCACATCAGAGTCATACAACCAGTTAACGATGGCAATCGTCTATACATTTGT
GGCACTAACGCACATAATCCCAAGGA
TTACATCATCTATTCTAATTTAACGCACATCTCACGGTCGGAGTATGTACCGGG
CATTGGACTGGGTATCGGTAAATGTC
CGTACGATCCACTGGACAACTCGACGGCCATTTATATTGAGCGAGGCAATCCT
GGAGATTTGCCGGCACTTTACTCGGGA
ACGAATGCGGAATTCACGAAGGCGGACACGGTGATCTTTAGGACCGATTTGTA
CAACATGACCTCGAAGACCAAGTCGTA
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TTGTCGGCTCCTTCGACGGTGGCGAGT
ACGTGTA

>c157132_g1_i1|m.35709
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VGSMDRVFKLNLENISTAACDRDQ
ILLEPTGSDVVNCVSKGKSQLFDCRNHIRVIQPVNDGNRLYICGTNAHNPKDYIIY
SNLTHISRSEYVPGIGLGIGKCPY
DPLDNSTAIYIERGNPGDLPALYSGTNAEFTKADTVIFRTDLYNMTSKTKSYNFKR
TLKYDSKWLDKPNFVGSFDGGEYV

c153060_g1_i1|m.2372

Effective counts for this sequence were notably high in adult male *vittatum* and adult post-oviposition female *vittatum* (Figure 2). Uniprot database matches indicate that the sequence codes for a guanine nucleotide-binding protein, or G protein, which are involved in signaling pathways. In addition to 'flight behaviour', the corresponding Gene Ontology annotations listed several functions the sequence could serve, such as 'regulation of feeding behaviour' and 'regulation of locomotion', alongside other receptor signaling pathways.

Effective counts:
Adult male *vittatum*: 1128.33
Adult nulliparous female *vittatum*: 349.4
Adult post-oviposition female *vittatum*: 2330.56
Female *tribulatum* larvae: 123.87
Male *tribulatum* larvae: 107.89

>c153060_g1_i1|m.23722
GTTGTTCAGTGTGGGCTTTTTATTTTAAAAAAACGTCAGAGGGTCGCGTCCAC
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ATGTAACTTCTTTTATCGGAGTGTTAATGATAATGAATTTTTAATCGATAAGAGT
ATCTTACACCGCCGTCCAACCAACC
AAACGAGTATGAGTACCTACACTTCAAAAACCCGACTTCAACCCGAGTCGGCA
GAAAAGTCTCCGATATCTTATCAATCG
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GCATTAATTGGCAAAGGACCAATTCA
TTAAACGCAAAAAAAAATCTGACAAATAACAACCTTATAACCGTGTGATTCACA
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AAGAGGTCTCACATCATATAGATCCAAAGTGATTATAGCGAGGTATAGTTGGTT
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ACATCAATTGAGATTAGCCGTTAAGA
AATCGATCAGAAATAAAATTTCCCGGAAATCGCAAACATCCCACTCTTACATAA
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CCTCAAAATCAACAAACCACGCTCATCGGCAACCTCGAATGCCTCCGATGCTT
CCAAAAAACTGGAAAAAGAGCTGACCA
AACGGACATATAAATTTGACAATGCTGTGAAAATCCTCCTGCTCGGCACCGGC
GAAAGCGGTAAAACTACGATATTGAAG
CAAATGAAGATACTGCACATGGAGAACGGATTCACGATGGAAGAACGACTCGC
GAACATCACCGCAATCCAGCTCAACAT
CCACGAGAGTATTTTCGAGATCTGTCGGAATGTCACGGTGATGGGCTTAGAGT
TCGACTCGCACACAAATCGAGAGAACG
CCAAGTGGATATTGTCGATGGGTCGTTTTGTGTACAATTTTTTCAGCAATGAGT
ATGTGGGCGCTGTAAAGGCGCTCTGG
GCGGATGCGGCCGTGCAACAATGTTTCATGAGAAAATCTGAATACCAGCTCAT
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AATTGATGAGATCAGTCTACCTGGCTTTGTGCCGAGCAATGAAGACATCCTCTT
GACCCGCAAAATGACGACCGGTATTC
GAGAGGTTACGTTTCAAGTGAAGATACCAAGCAGCATGGGCGGGGGTTTTCA
AGAGTTCCGTATGTTCGATGTGGGCGGA
CAACGTGATCAGCGCAACAAATGGATGCAGGCCTTTGAGGGCATTCAGGCCAT
CCTGTTCTTGATATCCTGCGGTGACTT
CGATCAAACTCTGCGTGAAGACCCTCAACAGAATCGACTCGCCGAGTCGATCA
AACTCTTCGACCGGGTCTGGCAGAATC
GATTTCTTTGCAGCGCAGGTGTCATCGTGTTTCTGAACAAACAGGACATCATG
GAGCAGAAGATTCGTGCGGGCAAAAAT
ATCGGCACATATTTCCCCGACTACTACCAATATAGGTTGTCTGCACAAGATGGT
AACGTGTTTGACGAGTTCAACAAAAC
GCGGTGTTTCATTCGCCAGCAGTTGGTTGAGGTGACTAAAGTGGTGCCACGTC
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CGAGAGAGTGCTTTTTTCACTTCACGGTGGCGACGGATACTCGTAATATCAAA
AAAGTGTTCAACGACGTCCACAATATC
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TCTCTGTACTAAATAAGATCTAAAACT
TTGCTTAAATGAAAAACTAAC

>c153060_g1_i1|m.23722
MVNNPMWRCLKINKPRSSATSNASDASKKLEKELTKRTYKFDNAVKILL
LGTGESGKTTILKQMKILHMENGFTMEERLA
NITAIQLNIHESIFEICRNVTVMGLEFDSHTNRENAKWILSMGRFVYNFF
SNEYVGAVKALWADAAVQQCFMRKSEYQLI
DSAKYFLDKIDEISLPGFVPSNEDILLTRKMTTGIREVTFQVKIPSSMGGG
FQEFRMFDVGGQRDQRNKWMQAFEGIQAI
LFLISCGDFDQTLREDPQQNRLAESIKLFDRVWQNRFLCSAGVIVFLNKQ
DIMEQKIRAGKNIGTYFPDYYQYRLSAQDG
NVFDEFNKTRCFIRQQLVEVTKVVPRRLSNIGREIPRECFFHFTVATDTRN
IKKVFNDVHNIILTRNLADMGLL*

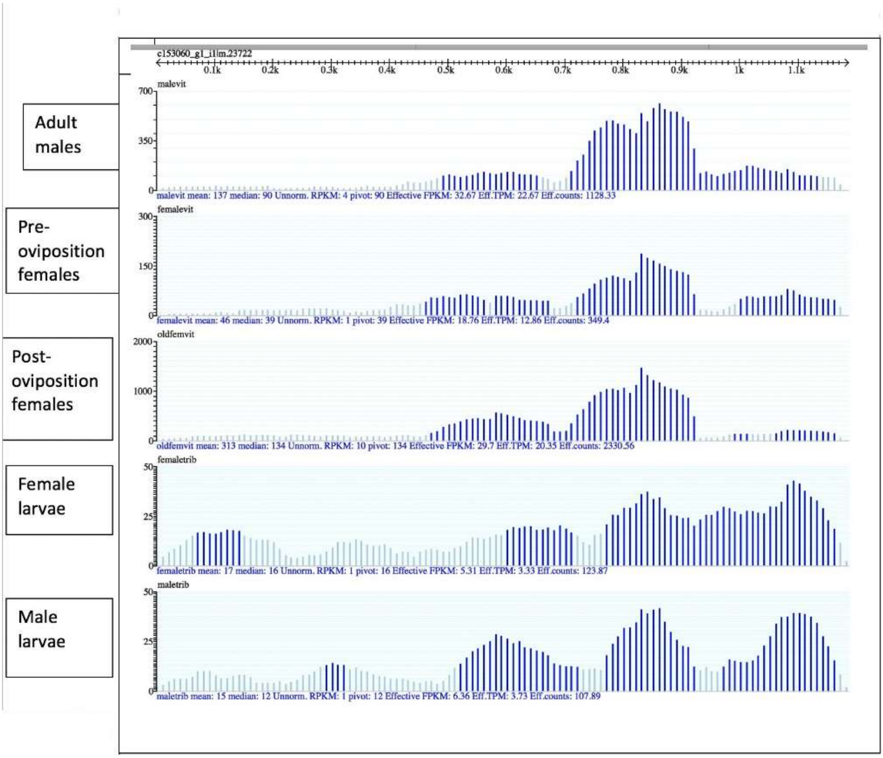

Figure 2. Coverage map showing the relative expression levels of "c153060_g1_i1|m.2372". The height of the bars indicate the number of reads mapped to that location along the gene, reflecting the amount of transcription of the gene. The X-axis indicates the length of the gene. Note that the Y-axis scale differs between developmental stages. This gene is most highly expressed in post-oviposition females, followed by adult males.

c162615_g1_i1|m.61334

This sequence follows the pattern of high expression in both adult male *vittatum* and adult parous female *vittatum*, though expression in the latter is somewhat higher than the former. One of its protein matches is protein flightless-1, which in *Drosophila* plays a structural role in indirect flight muscle. This protein may function similarly in blackflies.

Effective counts:
Adult male *vittatum*: 2742.08
Adult nulliparous female *vittatum*: 1020.63
Adult post-oviposition female *vittatum*: 4667.64
Female *tribulatum* larvae: 580.56
Male *tribulatum* larvae: 513.05

>c162615_g1_i1|m.61334
GTGAATATACCAAATCAGCTGAAACTTGAAAAAAAATAATTTTGTTTTGTTTAAA
TTTCTTTCTGAAAATTTCCCCTGAT
AAAAAGAGGCGATAAAACTGATAACTTCAAGCAAGAACGTAGCAATAAATGTC
ACGACTATAAAGTGATAGAAGTATTTG
CAATAATTGGCCGTTTGGGCATTACAATGAATTGTTGACCTCGAAAGTGCTTAA
ATTTTTTTCGTCCGTCTATTTTTGTT
TTAAAATATTACTAATTAATGCGTCTAATTCTGTCGTCACCCATATATCGTTAAT
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GTCGATTGTGGTGGCGACAGGAGCGAAGGGCGTTTGTGGAACGAACTGAAAG
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GAAGATCGACAATGCGTTCGAGTTCACGCCAATGTTGCAGCATCTGAATTTGA
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CGATCAGCACGTTGGCAGCGTTGCAGT
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GCGTCCGCGTATCTTCACAAGAATACG
TCCAGTGGAGTGCTTCTGCTCGACGGAACACAACTATCCAAGAGTGAAAAAGC
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CGCGCAGCAGTGTGGGCTCCCACAGCAGTTTGGTCATCGAGCATCTGGCCAA
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TCGTTGGTCCAACGTAAACGACCCTTTCGTCATATCACCCTCAAGGGTGACAC
ACCCGATGAGGCGGCCGTTGAAAAGCA
AAAGACGGTCGTTCGCAATCCCAGCACAGAGCACTTGCTGACCAAGAAAACCA
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CCAACTGGTTAAAAAGCAGGAATAAGGTGGGCAGTGTGTTAGGCTTCAAGAAT
TTGTCACCGGAGAAGTCGCCGTCAGTG
TTCGGCACCTCGCCCAGTGGTCTGAGCATCGTCAATCGGGCGCACGACAACTT
CGATGTCAGTGTCGTCACGTCAACGCC
CAAGGATCGAGATATTACCAGTAATTTTTCCCTGGAAGTTTCGTCGCCTGTTGG
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AGAGTGTATTGGCCACAACGGATGGAAGTGCGACTACGGATTATTTGTCGGCG
AATGAATCACCAACCACCAGGAATCGT

>c162615_g1_i1|m.61334
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KTIFLSLNLFMHDEPCDLIDISKFRNLRKIEVNRIAIDKVHGLKVLRPQMC
EVYCIRSLSCIEDILVDCGGDRSEGRLWN
ELKVADFSYNGLTKIDNAFEFTPMLQHLNLSNNRLVSVTAVKWLPNLKSL
NLNFNRLTEVPLLHADTCRRLQVLLIANNY
VEDLMGVACMDGLCELDLSMNCLLDHSTLLPISTLAALQYLNLKGNPMS
CHPKHRQVASAYLHKNTSSGVLLLDGTQLSK
SEKALTGNYASHRPKVMSQYQSNRSSMSSICSRATNDNTPRSSVGSHS
SLVIEHLANRSHGDDMNTSLVQRKRPFRHITL
KGDTPDEAAVEKQKTVVRNPSTEHLLTKKTIEEQAQKHGANWLKSRNK
VGSVLGFKNLSPEKSPSVFGTSPSGLSIVNRA
HDNFDVSVVTSTPKDRDITSNFSLEVSSPVGDVTTTTEYKSVLATTDGSA
TTDYLSANESPTTRNRQYSIFETIKNIQEA
PEVASDPEDNEKTYIVTEIDSAGKNLNEYILVISDKSLKEKNTETGRTQTR
WSLETLESCESMRSNTITLYFDTIRRDRK
ERTYRMSSPLEAKQLLNFLRNILSERDLAEMNQQVYNCAKCSVQFSREV
KPNMDPSCPECKSQFVIAMPTATVTKPAAEP
VAGVSKEQRDSPKRGMAKLWKSASHASIESAGSINDSQSSCSKISQSE
SSFDSNQSVAGSSDNSDRDKDIADLLRRGDGT

ESDDIEILSNPSQSSIEVLDTYYSNRKLSDERHILQRPSLETIDDEQQQIA
NLSTTLTNRETSTSDLNLSALNREIAEMA
KSGEKIDPVEGATKEDKKDKVHKKSGISGGLLLTESSSSGSVTDSVCTA
YEQNQMVKSDGSDAATPKKDEAKHTNGKAEN
VSVITTMLGGLFQSTNLLMAKTPKTPSKPDLLMAGQPEPYRYSFTDFTAV
DHRLKLFFFQSVFEDDGELMNWLVRGRLVD
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RLCILKVLPWKMGLTLTIKSFGVIHLLLPDIS
RTDSLLLFLADNPLPHTCRLDYQVSDTANQKLQALTSQKQIKSLTIFNEC
RISAYEQHQINDSGCLCVTDTDLFLLQLDT
NWLNDTINAPITVTNRQEMANLIEAEVVDAVTFKLNFLNESEDRYEMWK
VTFDATETAEATIQTISNWWEKIFGVPLIGG
HHVMMETSGVAVS*

c41610_g1_i1|m.1164

Effective counts for this sequence show highest expression in adult post-oviposition female *vittatum*. The Uniprot database matches are kinases, and the Gene Ontology annotations indicate a role in transcription regulation.

Effective counts:
Adult male *vittatum*: 24.26
Adult nulliparous female *vittatum*: 3.15
Adult post-oviposition female *vittatum*: 102.09
Female *tribulatum* larvae: 46.68
Male *tribulatum* larvae: 16.62

>c41610_g1_i1|m.1164
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CGTGGGCACGCCGTGCTGGATGGCTCCGGAGGTGATGGAGCAGGACCATGG
GTACGACTTCAAGGCCGACATCTGGTCGT
TTGGCATAACGGCCATCGAGATGGCGACGGGTACGGCGCCCTACCACAAGTA
CCCGCCGATGAAAGTGCTGATGCTGACG
CTGCAGAACGACCCGCCCACCTTGGATACGGGCGCCGACGAGAAGGACCAGT
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>c41610_g1_i1|m.1164
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LQNDPPTLDTGADEKDQYKAYGKTFRKLVTECLQKEASKRPSANELLKH
QI

c157939_g1_i1|m.38649

Expression levels of this sequence are highest in adult post-oviposition female *vittatum*. Activated and cyclin-dependent kinases are its most frequent protein matches.

Effective counts:
Adult male *vittatum*: 26.61
Adult nulliparous female *vittatum*: 105.5
Adult post-oviposition female *vittatum*: 851.34
Female *tribulatum* larvae: 204.78
Male *tribulatum* larvae: 221.15

>c157939_g1_i1|m.38649
GTCAGCGCTGTTAGCATTTTAACTGAAATTTTAAAAATCGCAAAAGTCAAAACA
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ATATCCTGCAAACACCTCTACGACTT
CCGAAATCACCATTGCTAGAGCGTCTAGGTTGCGGCACTGGCGTCGAAGTGAT
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AAACTTCGCCTCGCCCTGGGCTATCAAACGCATCTCCAAGCGACAATTGACCA
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GGGCCCCTTGCCCGTCGAACTGACGATGAAAATGGTGTTGGACGTCGCCCGG
GCACTGGAGTATTTGCACACCAAGGCGT
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AGGTGTGTAAGTTGTGCGACTTTGGC
GTCAGCCTTCCATTGGACGCCGAAGGCTTCGTCGACACCGATAAGTCACCCGA
CGCTCAATATGTCGGGACACCGTTGTG
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GACATCTTCTCCTTCGGCATGGTCATCT
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AGTACGAACAGATCATTGAGTTGTTTTTCATTTGCACCTGTGAGGAGTCGGAC
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AAAGCGTTGGAGTAGTGATGGATAAGGTTTTAGTTTTGCAATTTTTAAACGAA
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GGCTACCGATACCCAAATCAAGATGCAGGTTCGTTTCTGAAAGTGAAATTAAA
AAGAAATTAATAAAGTCCATAGAATGG

>c157939_g1_i1|m.38649
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VELTMKMVLDVARALEYLHTKALL
LHGDLKSFNVLVKREFEVCKLCDFGVSLPLDAEGFVDTDKSPDAQYVGTPLWSAP
EVFEEEIELITTKCDIFSFGMVIYE
TMALIPPHTQQLHLADDATSSVISLDDTVGGKKDYDENGDSIIVLDDSVDLESSA
KNITLTECYGKRPPIPDTVADMKEY
EQIIELFFICTCEESDERPSASDLVKALE*

c145375_g1_i1|m.12724

This sequence had very high effective counts in adult post-oviposition females. All of the protein matches were ubiquitin-conj'ugating enzymes. Gene Ontology annotations gave a wide variety of possible functions, such as muscle degradation", "determination of adult lifespan", "mitotic spindle organization", "UV protection", and "apoptosis regulation".

Effective counts:
Adult male *vittatum*: 126.39
Adult nulliparous female *vittatum*: 201.28
Adult post-oviposition female *vittatum*: 1004.01
Female *tribulatum* larvae: 103.57
Male *tribulatum* larvae: 97.28

>c145375_g1_i1|m.12724
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ACCAGATATAAGAACAAAACGAGTTT
CAATTGAAATTGAAAAAATAAATAAGGGAACTGGCAGCCATGGAATATCGATC
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CAAGATGAGCATTGACATTTGTCCCAG
ATACCCATTTGAGCCTCCGTTATTCCGTTTCCTTTCACCTGTGCCTTACCATCCT
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GTTTGGATGTGTTGCGGTTGCCGCCTGGCACCTACAATCCTGCTATCACACTG
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TTGCTTTCCAATCCAAACCCAGATGATCCACTGCGAGCTGAAGCTGCCGATGA
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>c145375_g1_i1|m.12724
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YHPNIDESGHICLDVLRLPPGTYNPAITLESILISVQLLLSNPNPDDPLRAE
AADEFRYNPILFAKKASNFV*

c156338_g1_i1|m.32945

Although all three adult groups have high effective counts for this sequence, the adult parous female count is the greatest by far (Fig. 3). All of the Uniprot matches were G proteins, and the Gene Ontology annotations, such as 'rhabdomere', 'retina development in camera-type eye,' and 'phototransduction', suggest that the protein plays a role in visual signaling.

Effective counts:
Adult male *vittatum*: 2416.73
Adult nulliparous female *vittatum*: 2024.74
Adult post-oviposition female *vittatum*: 3951.08
Female *tribulatum* larvae: 241.11
Male *tribulatum* larvae: 274.28

>c156338_g1_i1|m.32945
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ACAAGTTCCTGAATAAGGATAAAAGTGTGCTACGACGGCAGGTCAAGCTCCTT
CTCCTCGGCGCCGGTGAATCCGGCAAG
TCCACCTTCCTGAAACAGATGCGCATCATCCACGGCGTCAAGTTCGAGCCCGA
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CTACCAAAACATCGTCAAAGGTATGCAAGTTCTAGTCGACGCCCGGGAAAAAT
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TCAAGTGCTGGCCGAGGACAGAAAAACCAATCGGCTGGAAGAATCGAAAAAC
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CGACACCGAAAACATTCAAGTGGTCT
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CAGGAATTTCGAGATATGCGAACCAA
GCGAGTGAGATAATTTAGAAGAAAATCTACAAGTTTTTCGTTCTATGTTGTTAT
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>c156338_g1_i1|m.32945
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PFVFVDVGGQRTQRQKWTKCFDTS

VTSIIFLVSTSEFDQVLAEDRKTNRLEESKNIFDTIINNTAFKGISIILFLNKTDLLA
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MLQ*

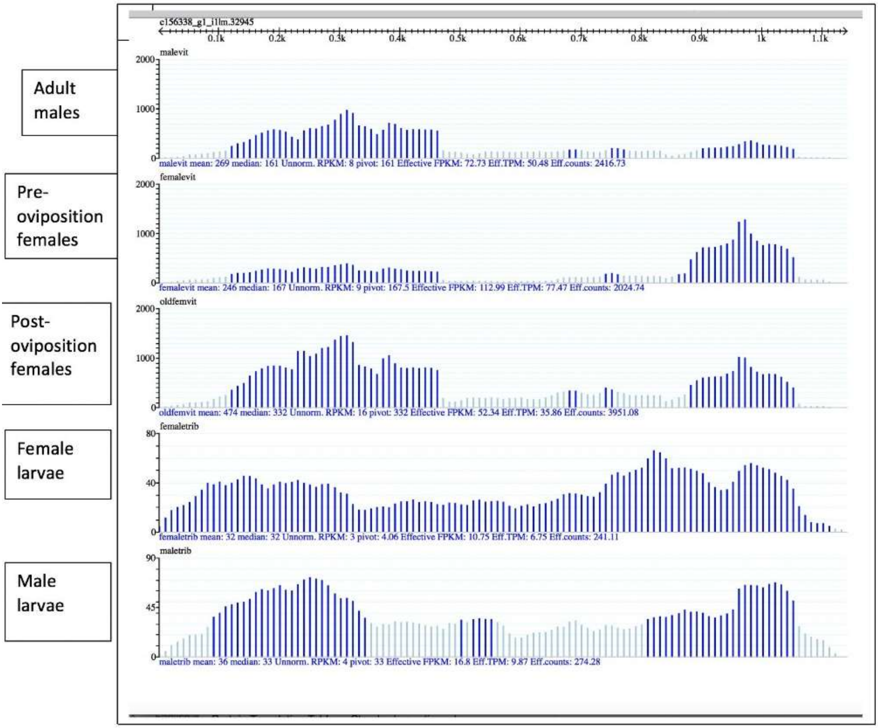

Figure 3. Coverage map showing the relative expression levels of "56338_g1_i1|m.32945". The gene is heavily transcribed in all adult stages, with lower but significant expression in larvae.

c151900_g2_i1|m.21264

Several different proteins matched to this sequence in Uniprot, including Activin receptor, TGF-beta receptor, cell division control protein, cyclin-dependent kinase, mitogen-activated protein kinase, and bone morphogenetic protein receptor. Both these protein matches and the Gene Ontology annotations suggest that the protein functions in a regenerative pathway. This sequence was also in keeping with the pattern of high expression in adult male *vittatum* and adult parous female *vittatum*.

Effective counts:
Adult male *vittatum*: 386
Adult nulliparous female *vittatum*: 8.7
Adult post-oviposition female *vittatum*: 62
Female *tribulatum* larvae: 22.71
Male *tribulatum* larvae: 76.09

>c151900_g2_i1|m.21264
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GACAACAGCACCTCGTTCGCCATCTTTTGCAAAATGGCCATATCCATAGCTTCA
GGCCTAGCCCACTTGCACACTGAGAT
CCGAAAGGGTGACCAGTTTAAGCCATGCATCGTCCACCGCGACCTGAACTCGC
GGAACATTTTAGTCCGTCCGGACTTGT
CGTGCTGCATCTGCGACCTGGGCTTCTCGATGAAGGTGTACGGGCCCAAATAC
GAATACCGCGGCGAGATCAACCTGGCC
GAGACGAAGAGCATCAATGAGGTGGGTACGTTGCGTTACCATGCGCCCGAGG
TCCTCGAAGGTGCCGTCAACCTTCGCGA
TTGCGAATCGAGCTTAAAACAAATCGACATATACGCCATGGGCTTGGTCCTGT
GGGAGCTTTGCACACGTTGCCACGATT
TTTACTACGCGTCTGAAAAGCTGCCGCCACCCTACAAAGCCCCCTATGAGGCT
GAAATCGGCTGCAATCCAACGTTCGAG
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CCGAACACGCTCAAAGAGATCGCCTCTGTTCTCGCCCCGCCCAATCACACGGC
TCCCGACATGACGGCCAGCGAGCCACC
GCAACAGCTCAAAAACCGCGAAATGTTCTCGCACCAAATCCAGGCATTTCAGG
GCCGTAATCCAACAATGGAGCGCAATC
TGGTTCAGCCTGCTGAGAAACAGCCAGCACTGGTGGCGAAGAGTAAAAAGCA
CGCTGACCCTCAGAAGATGAACAACAAC
GACAACGAGCGACTGAACGAGAATTTTATCATCGACGAGCTGATGAATGCGCC
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CAAGAAAATCCAAAATGTCGAGAGCACACGGACCAAGGGATGGCAGAGTGTA
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ATCGAACCCCACCCGGTGAGAAAACCGTACAGAAATTCTCCGTCATCAATCCA
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AGAAAACGTTGTCATTGTATGACGACCGA
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AGGCATTGGAATAAGACCAC

>c151900_g2_i1|m.21264
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EPPQQLKNREMFSHQIQAFQGRNPTMERNL
VQPAEKQPALVAKSKKHADPQKMNNNDNERLNENFIIDELMNAPATSM
SEGFSKKIQNVESTRTKGWQSVRNLLNKKFFR
KPDAYHFHCDEKSNLVDNRSKLVYNVNVENGAYSDDVTTSPVTDHPTN
GVALRPKNLDISPIVVKKFDQQQTHNGESSAY
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FLKRQRSLEVFREVFGPKGSVERLRDPSQR
VKTPGDVPPSVRKVRAKKTLSLYDDRMMDSGTMAAQYGV*

c162153_g1_i1|m.58651

For this sequence, the effective counts in adult male *vittatum* were notably higher than any other group (Fig. 4). The single Uniprot database match was protein Turtle, a protein known to play a role in coordinated motor control and axonal targeting of the R7 photoreceptor in Drosophila. It could be that this sequence codes for a protein of similar function in blackflies. This sequence's Gene Ontology annotations appear to support this idea, with top listed matches of 'adult locomotory behaviour', 'axon guidance', 'flight behaviour', and 'synaptic target recognition'.

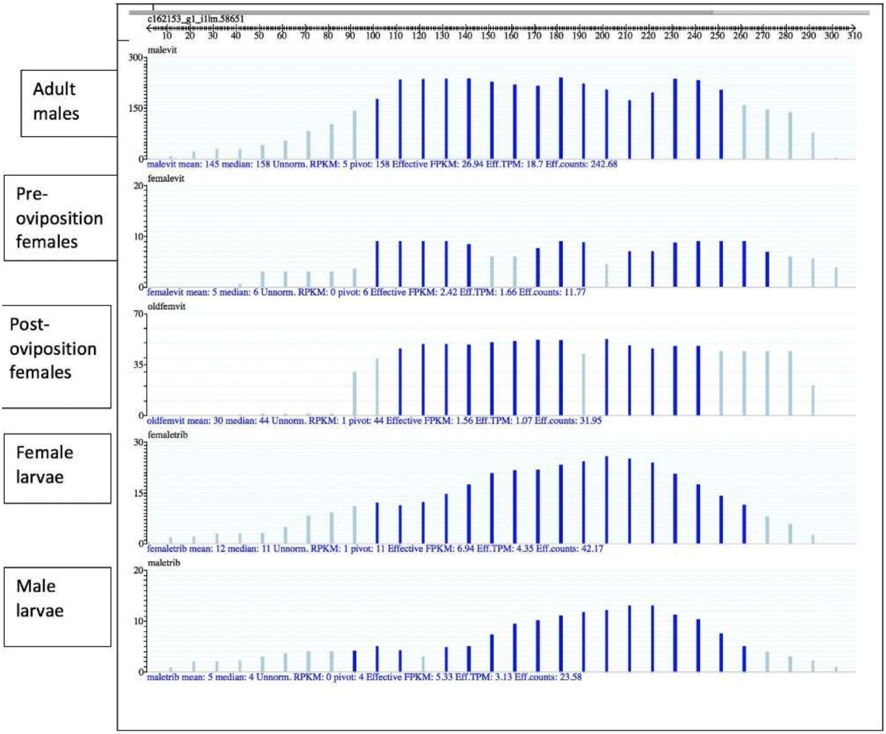

Figure 4. Coverage map showing the relative expression levels of "c162153_g1_i1|m.58651". The expression of this gene is highest in adult males.

Effective counts:
Adult male *vittatum*: 242.68
Adult nulliparous female *vittatum*: 11.77
Adult post-oviposition female *vittatum*: 31.95
Female *tribulatum* larvae: 42.17
Male *tribulatum* larvae: 23.58

>c162153_g1_i1|m.58651
CGATCTCTCGGGCGGCACCGCCAACAACGAATGGCGCTACCTGCCGCCCTAC
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TCCAGTACTACCAAAACCACGGCTATCACTTGCAACCCCCCACACCGCCCACT
GTGGGCCACTGGTTGGACCTGATCGCC

CGCCTCAACTCCGCCACCGACAAAGGCGGCATCGTCAAGAAGGCCATCGATG
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GCGACGACATCGACGCCTC

>c162153_g1_i1|m.58651
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DPATPTPSASTPTGVYLRDDIDA

c151982_g1_i1|m.21390

While expression for this sequence was high across the board, it was especially so in adult male *vittatum* and adult parous female *vittatum*. Protein matches include spectrin beta chain, alpha-actinin, nesprin, and a nuclear anchorage protein. Gene Ontology annotations suggest this is a cytoskeletal anchoring protein.

Effective counts:
Adult male *vittatum*: 1357.08
Adult nulliparous female *vittatum*: 517.25
Adult post-oviposition female *vittatum*: 1166.61
Female *tribulatum* larvae: 319.79
Male *tribulatum* larvae: 142.25

>c151982_g1_i1|m.21390
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GAAAATCGAAAGAACTTCAAACATA
TTAAGATTACGAAGTCAAAAAACGATGATAAAAAAAAACAGTTAAAACACCATT
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MLAEVLAYWEKWQVISAEFLHYLNEA
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RETILQQLDQHKHFFGRIVYYKSM
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TISWKGFIDCERNLSNWFRHAEHYFQ
NISSTSTANDLEIQKQFFDSFDERWLQEFESCTADLIKLSSISVQMTNCSVGNNIL
QRVESIRNLDSATKLKIPIHMLRL
EYTKNQEV

c139920_g1_i1|m.9473

The highest expression for this sequence was in adult post-oviposition female *vittatum*, followed by adult male *vittatum*. Protein matches include spectrin alpha chain, alpha-actinin, and an uncharacterized protein C50C3.2 that has been identified in C. elegans.

Effective counts:
Adult male *vittatum*: 3878.23
Adult nulliparous female *vittatum*: 1035.31
Adult post-oviposition female *vittatum*: 5883.53
Female *tribulatum* larvae: 1384.54
Male *tribulatum* larvae: 1358.75

>c139920_g1_i1|m.9473
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NDPKTGHPVTGALDYVD

References:

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