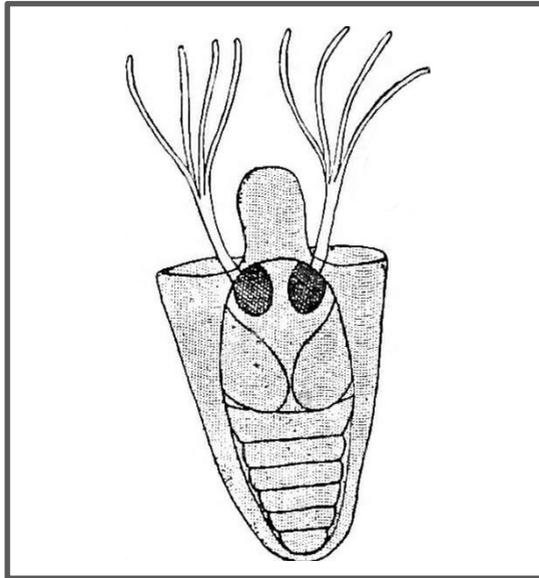


# *The Simuliid Bulletin*

Number 55

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# THE SIMULIID BULLETIN

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***Cover Image:*** From page 504 in Chandler, A.C. "Animal Parasites  
and human disease" (1922), J. Wiley, New York.

## **From the Editor**

I am beginning this editorial with the sad news. In 2020 the simuliid community lost two exceptional members and inspiring researchers - Dr. Doug Craig and Dr. Monty Wood, who exceedingly contributed to simuliidology with their lifelong work. The current issue brings a tribute to the excellent dipterologist Dr. Donald Montgomery Wood.

Besides sharing scientific papers, the Simuliid Bulletin informs about the planned meetings of the simuliid community, so you can mark your calendar and not miss the opportunity to share your ideas. Unfortunately, this time the current issue of the Simuliid Bulletin does not contain the Forthcoming meeting section. At the beginning of the year 2021, we still do not know when we will have the opportunity to meet.

We will inform you at the Simuliid Bulletin webpage if the situation changes. Of course, we still invite you to share your papers, ideas, short communications, or remarkable photos on the pages of the bulletin.

Stay safe and healthy.

Tatiana Kúdelová, Editor

## OBITUARIES

### **Tribute to a Naturalist and Systematist Nonpareil: Donald Montgomery “Monty” Wood (1933–2020)**

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The Chickasawhatchee River—type locality of *Simulium taxodium*—was in its spring flood stage, running high above its banks. Unable to see the riverbed or maintain our balance in the swift current, we watched as Monty, standing nearly 2 m tall, nonchalantly waded to the fallen tree near the river’s middle. With a small saw attached to his belt, he cut off vegetation with hundreds of larvae and pupae and returned them to the shore. Monty’s commanding stature and deep interest in understanding the natural world often meant he was undaunted by difficulties in the field. But, he prepared well, and when he was near civilization, he knew where to find the best dining establishments, and always insisted on treating his younger traveling companions to an excellent meal.

Monty Wood’s place in dipterology is set in gold among the past giants, and other tributes are likely to be written. We take this opportunity to highlight his contributions to simuliidology in the larger context of his life and profession. On a personal level, Monty had a profound influence on both of us and it was a privilege to have worked and traveled with him over many years.

Donald Montgomery Wood was born on 22 December 1933 in London, Ontario, Canada. At the age of 5, he began making natural history collections. He had a special fondness for his early collections of insects, particularly beetles, and bird eggs and held them into the 21<sup>st</sup> century. He also was an ardent birdwatcher and a talented botanist. More broadly, if it moved or grew, Monty had some knowledge of the organism.

Monty received his B.A. in Honors Biology (1956) and his M.A. (1959) in Parasitology from the University of Toronto. While an undergraduate student, he worked in Algonquin Park, Ontario, as a summer research assistant with the parasitologist Albert Murray Fallis (1907–2003) of the

Ontario Research Foundation, Toronto. He captured biting midges (Ceratopogonidae) and black flies feeding on ducks, so they could be screened for infection by avian blood parasites (*Haemoproteus*). His second publication (Fallis & Wood 1957), and the first involving black flies, resulted from this research. It was Monty's introduction to the broadly defined genus *Eusimulium*, which would later command the attention of his doctoral work. But in the interim, he chose to study the ectoparasitic beetles of beavers (*Castor canadensis*) for his Master's research (Wood 1959).

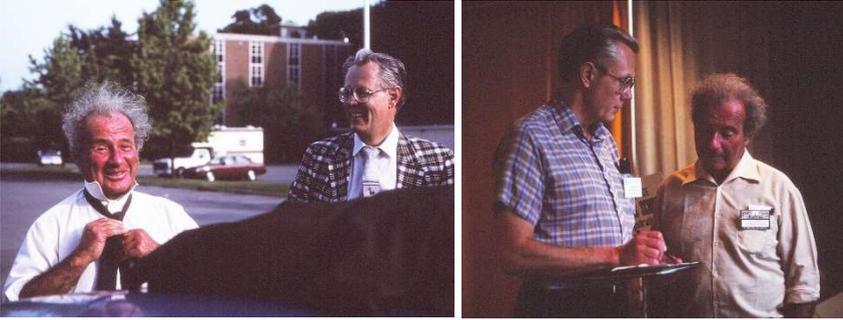


**Fig. 1.** Monty Wood and his doctoral advisor, Douglas Davies, in February 1996 at the annual meeting of the NE-118 Regional Black Fly Technical Committee in Flamingo (Everglades National Park), Florida, 33 years after Monty received his Ph.D. at McMaster University.

Monty was intrigued with the northern, small-stream black flies, most then assigned to the subgenus *Eusimulium*. They were the focus of his doctoral research at McMaster University in Hamilton, Ontario, under the guidance of Douglas M. Davies (1919–2008) (Fig. 1). Monty often collected his research material along Ontario's roads from Hamilton to Algonquin Park in an exotic Citroën sedan with handy features for fieldwork, such as a suspension system that could be raised by the driver when traveling over rough terrain (*in litt.* S. M.

Smith to PHA, 4 October 2020). Monty perceptively divided the species of *Eusimulium* into eight groups based on morphological characters (Wood 1963a), six of which would come to represent separate subgenera and the other two to represent species groups, all in the genus *Simulium*. Monty's early appreciation of cryptic species was spurred by his association with Klaus H. Rothfels (1919–1986) and a contemporary doctoral student, Robert W. Dunbar (1930– ), working in Rothfels' laboratory at the University of Toronto. In later years, Monty told us that Rothfels had been his most important mentor (Fig. 2). While sweeping adults of *Simulium* (*H.*) *congaenarum* from blueberry swales in Ontario, he noticed that the forecoxae of some of the females were yellow and some were gray. Pursuing the difference, bolstered by Dunbar's (1962) chromosomal work, he described a new species, *S. anatinum* (Wood 1963b). While a doctoral student, Monty also worked with fellow doctoral student James K. Lowther, an ornithologist at the University of Toronto, to describe the remarkable attraction of *S. annulus* to the common loon (*Gavia immer*), suggesting that the attraction was mediated by olfactory and visual cues (Lowther &

Wood 1964; as *S. euryadminiculum*).



**Fig. 2.** Monty Wood and Klaus Rothfels at the International Black Fly Conference, Pennsylvania State University, 29 May 1985. **A.** Monty looking on with amusement as Klaus uncomfortably applies a necktie—required attire to attend the conference’s evening banquet. Neither Monty nor Klaus was comfortable in a suitcoat and tie, much preferring the casual garb of field and laboratory. **B.** Monty and Klaus discussing the origin of the name *Simulium decolletum* (currently a synonym of *S. fontinale*). The name had been coined (as “décolleté”) during a July 1982 black fly expedition to the Yukon by Monty, Klaus, and Roger Crosskey.

During his doctoral studies, he met a young student, Grace, in a biology class for which he was the teaching assistant. They subsequently spent much time together, practically living in Douglas Davies’ first-floor corner laboratory in McMaster’s Hamilton Hall, cooking their meals together on Bunsen burners (*in litt.* S. M. Smith to PHA, 4 October 2020). In 1963, Monty received his Ph.D., and in the latter half of that summer, shortly after their marriage, they traveled the highways of Alaska and the Yukon on a major collecting expedition for black flies and other insects. In the spring of 1964, they collected in the Pacific Northwest, particularly British Columbia. Much of the western material that they collected was used in cytological studies of *Stegopterna* and *Prosimulium* (Ottonen 1966, Madahar 1969) and a revision of the *Prosimulium* of Canada and Alaska (Peterson 1970). Grace and Monty remained in a devoted marriage for 57 years and raised a son (Kenneth) and a daughter (Sheila).

Upon graduation with his doctoral degree, Monty received a one-year postdoctoral research fellowship from the National Research Council of Canada. In October 1963, he and Grace went to Ottawa so that Monty could pursue his postdoc on the black flies of Canada with Guy E. Shewell (1913–1996) and Bobbie V. Peterson (1928–2006). Peterson had been temporarily transferred from the Medical and Veterinary Entomology Research Laboratory in Guelph to Agriculture Canada in Ottawa to work on the project for a year. Near the end of 1964, however, Agriculture Canada closed the Guelph lab and had to find positions for its employees. By August 1964, Monty was anxious to find permanent employment and went to the Federal Forestry Department to see if they might want him to work on the Tachinidae, a challenging group in which he became interested while

an undergraduate student. The next day, Eugene G. Munroe (1919–2008) was sent by the Director of Entomology to inform Monty that the Forestry Department felt it would be more appropriate for the position to be in Agriculture with the Canadian National Collection (CNC). The CNC, however, needed to take Peterson whose experience was with the Simuliidae. Monty, whose expertise was with both the Simuliidae and Tachinidae, therefore, was hired to work on the Tachinidae, with permission to work on the simuliid material he and Grace had collected in the Yukon and northwestern Canada. The simuliids that Monty and Grace had collected in British Columbia and the northwestern United States in the spring of 1964 had to be turned over to Peterson (*in litt.* Grace Wood to PHA, 8 January 2021).



**Fig. 3.** Monteverde, Costa Rica, February 2002. **A.** Monty Wood teaching students in the University of Toronto's Tropical Ecology and Evolution course at the Biological Station. **B.** Monty and Grace Wood.

Thus, on 1 October 1964, Monty was formally appointed to the Diptera Unit in the Entomology Research Institute with Canada's Department of Agriculture in Ottawa. For a while, he served as Head of the Diptera Section. Monty also was deeply involved in the production of the three-volume *Manual of Nearctic Diptera*, a long-term project in which he participated from its inception in the 1960s, and which he led from 1978 to 1981 (Cumming et al. 2011). After retirement in 1986, he continued as an Honorary Research Associate with Agriculture Canada. He also continued his appointment as an adjunct professor with Carleton University, which began in 1970, teaching courses on Diptera, Phylogeny of Diptera, and Advanced Insect Systematics, as well as his famous tropical entomology field course at the Monteverde Biological Station in Costa Rica (Fig. 3).

Monty was happiest when he was in the field (Fig. 4). He explored some of the most distant areas of the globe, from the arctic tundra to the Australian outback and from the tropical rain forests to 4,000 m above seal level in the Andes Mountains. He and Grace spent the summer of 1975 camping in the Kuujjua River Valley on Victoria Island in the Canadian



**Fig. 4.** Monty Wood ascending the hills above Brasstown Falls, South Carolina, to search for hilltopping black flies and tachinids, 15 April 1989.

Arctic Archipelago to collect insects such as simuliids and tachinids, with a trunk of books for reading material in the desolate, treeless landscape. He purchased land in the cloud forest near Monteverde, Costa Rica, and in 1991 played a major role in building a biological station for research and instruction, complete with dining and sleeping accommodations and computer and laboratory space.

Monty was as clever as he was dedicated in the realm of natural history. He discovered that adults of species of *Greniera* and *Helodon* could be reared from last-instar larvae by placing them in petri dishes with a thin covering of water and leaving them on the door of his refrigerator. Sporadic opening of the refrigerator was sufficient to aerate the cold water and produce pupae. He stuffed plastic garbage bags into streambeds and later retrieved them to obtain pupae of prosimuliines and *Greniera*. Monty also developed an underappreciated technique for cross-breeding different species of black flies. He found that he could accomplish fertilization by dissecting

mature eggs from *Simulium bracteatum* (as *S. aureum* A) and mixing them gently in a small watch glass with the spermathecal contents from *Simulium pilosum* (as *S. aureum* B) (Wood 1963a).

For many years, Monty and Grace drove a large, three-quarter ton, green van (Ford Econoline F250), refitted with living quarters and collecting equipment and complete with the essentials of a field lab, including microscopes, insect drawers, binoculars, and plant presses. He learned and developed numerous techniques for rearing black flies (Wood & Davies 1966). A master of producing superb pinned specimens, Monty individually reared thousands of simuliids from pupae by placing them in vials with a sprig of sphagnum moss, and allowing the subsequent adults to tan and harden in the dark at room temperature for 24 hours. He dispatched the flies by freezing them, affixed them and their dried exuviae to an insect pin with a spot of shellac gel on the right side of their thorax, and freeze-dried them in a deep freeze for 4–6 months (Hunter et al. 1994). His specimens now reside in the Canadian National Collection.

Monty also had a keen interest in the fanless prosimuliines. He long argued—beginning in the early 1960s—that labral fans were present in the ancestor of all black flies, and their absence in groups, such as *Gymnopsis* and *Twinnia*, was a derived condition (Wood 1963a). His hypothesis went against the ideas of most of the biggest names in simuliidology at the time, such as Ivan Rubtsov (1956) and Lewis Davies (1965), who argued that the fanless condition was ancestral. The evidence, bolstered by Doug Craig's (1974) detailed morphological work and subsequent cladistic and molecular analyses (Currie 1988, Moulton 2003), has weighed in favor of Monty's hypothesis, and his idea is now widely accepted. Monty Wood's (1978) paper on the taxonomy of the Nearctic *Gymnopsis* and *Twinnia* and his insights into the ancestry of the Simuliidae stand out as one of his best works and a classic among the 10,000-plus papers that constitute the simuliid literature. The paper illustrates the power of a perceptive mind, the capacity to transcend established dogma, the benefits of fieldwork, the ability to merge minute details with broad conceptual thinking, and the talent of skillful writing.

He was the first to discover females of the genus *Parasimulium*, and by obtaining eggs from a gravid female in 1983, he also was the first to discover the larvae, rearing them to the second instar in a vial with distilled water in a refrigerator (Borkent & Wood 1986). He spent time searching for the elusive larvae and pupae in the Pacific Northwest of the United States, at one time considering the possibility that they were phoretic on tailed frogs (*Ascaphus truei*).

In the 1980s, Monty decided to begin spending the majority of his time on the Tachinidae rather than the Simuliidae. The decision independently coincided with that of the world's only other person working on both families—Roger W. Crosskey (1930–2017)—who decided to move in the opposite direction (Adler 2018). Monty was not simply the world's leading authority on the Tachinidae. He was a phenomenon. Nearly 10,000 species of tachinids have been described globally (Courtney et al. 2017), and it would not be an overstatement to claim that Monty was familiar with most of them. How many people placed boxes of tachinids before him for identification is hard to know. But, he could identify them and provide their natural history as fast as one could record the information, and if a stray calliphorid or rhinophorid was in the box, you received the species name for it, too.

Nonetheless, after signing a contract with Cornell University Press on 17 October 1989 to produce a book on North American black flies (Adler et al. 2004), Monty found a renewed interest and vigor in the Simuliidae. He began collecting over much of the North American continent, visiting type localities and little-collected areas throughout the 1990s—one moment in the Canadian Maritimes, the next in the mountains of southern Arizona, then on to remote areas of Alaska and the Yukon or to the Balcones Escarpment of Texas. In the late 1990s, the tachinids began tugging on

him again, particularly the tachinids of Costa Rica, as did the increasing demands for his expertise with these flies. As Monty put it, "...while I could collect simuliids, and get enthusiastic about them while doing so, when it came to studying them for characters, my tachinid work, which was always ongoing...and has taken precedence for the last 35 years, always seemed to interfere... Now I have virtually forgotten what I ever knew, or at least feel as if I have forgotten everything, at least how to tell them [simuliids] apart. It seems to be a matter of training the brain, and my brain has been trained to spot useful details on a tachinid body, and to recognize thousands of profiles and bristle arrangements" (*in litt.* to PHA, 9 February 2000). By the time *The Black Flies (Simuliidae) of North America* was published in 2004 (Fig. 5), Monty had moved entirely to the Tachinidae. Most of his published work on the Simuliidae, therefore, appeared before the 1990s.



**Fig. 5.** First opening of the *Black Flies (Simuliidae) of North America* by authors Peter Adler, Doug Currie, and Monty Wood at the Canadian National Collection, Ottawa, 21 April 2004.

We would be remiss if we did not mention that in addition to the Simuliidae and Tachinidae, Monty was proficient with other families of flies. Perhaps most notable was Monty's knowledge of mosquitoes. He was the lead author of the now-classic "The Mosquitoes of Canada (Diptera: Culicidae)", which treated the identification of larvae and adults, bionomics, and distributions of all 74 species known at the time from the country (Wood et

al. 1979). He also contributed chapters on four families other than the Tachinidae in the *Manual of Nearctic Diptera*: Axymidae, Pachyneuridae, Rhinophoridae, and Oestridae.

Among his more than 75 lifetime publications, 20 dealt in some respect with the Simuliidae (listed below). He was an author or coauthor of 57 species names in the Simuliidae. More than 30 species were named in his honor, including one in the Simuliidae, honoring both Monty and Grace: *Prosimulium woodorum* Peterson, currently a synonym of *P. shewelli* Peterson & DeFoliart.

Perhaps Monty's greatest contributions were to the young people he mentored—the students and incipient professionals who shared his deep interest in the natural world. He traveled often and nearly always included a visit with a graduate student or a young colleague, guiding them in the

nuances of dipteran systematics, sharing his remarkable insights into the natural world and offering genuine encouragement. Just a month before his 77<sup>th</sup> birthday and after a number of health setbacks, including triple bypass surgery, he wrote: "What keeps me going is the tremendous involvement with people around me" (*in litt.* to PHA, 23 November 2010). In typical humble fashion, what he did not realize was that it was his energy and his enthusiasm for the living world that inspired so many of us. Monty was inspiration writ large. His big laugh always affirmed that you were in the company of a good friend.

Monty was still actively working on his beloved flies when he died at his home, age 86, on 24 August 2020.

## Acknowledgments

We thank Grace Wood for reading the manuscript and providing the details of Monty's arrival and early assignments in Ottawa, Steve Smith for sharing his reminiscences of Monty's graduate student years, and Al Wheeler for useful comments on an earlier draft.

## References

- Adler, P. H. 2018. Roger Ward Crosskey—The life and contributions of an entomologist par excellence: (1930–2017). *Zootaxa* 4455: 35–67.
- Adler, P. H., D. C. Currie & D. M. Wood. 2004. *The Black Flies (Simuliidae) of North America*. Cornell University Press, Ithaca, NY. xv + 941 pp. + 24 color plates.
- Borkent, A. & D. M. Wood. 1986. The first and second larval instars and the egg of *Parasimulium stonei* Peterson (Diptera: Simuliidae). *Proceedings of the Entomological Society of Washington* 88: 287–296.
- Courtney, G. W., T. Pape, J. H. Skevington & B. J. Sinclair. 2017. Biodiversity of Diptera. Pp. 229–278. *In* R. G. Foottit & P. H. Adler (eds.), *Insect Biodiversity: Science and Society*, Volume I, 2<sup>nd</sup> edition. Wiley-Blackwell, Chichester, UK.
- Craig, D. A. 1974. The labrum and cephalic fans of larval Simuliidae (Diptera: Nematocera). *Canadian Journal of Zoology* 52: 133–159.
- Cumming, J. M., B. J. Sinclair, S. E. Brooks, J. E. O'Hara & J. H. Skevington. 2011. The history of dipterology at the Canadian National Collection of Insects, with special reference to the *Manual of Nearctic Diptera*. *Canadian Entomologist* 143: 539–577.
- Currie, D. C. 1988. Morphology and systematics of primitive Simuliidae (Diptera: Culicomorpha). Ph. D. thesis. University of Alberta, Edmonton, Alberta. 331 pp.
- Davies, L. 1965. The structure of certain atypical Simuliidae (Diptera) in relation to evolution within the family, and the erection of a new genus for the Crozet Island black-fly. *Proceedings of the Linnean Society of London* 176: 159–180.
- Dunbar, R. W. 1962. Cytotaxonomic studies in Simuliidae. Ph. D. thesis. University of Toronto, Toronto, Ontario. 101 pp.
- Hunter, F. F., S. G. Burgin & D. M. Wood. 1994. New techniques for rearing black flies from pupae (Diptera: Simuliidae). *Journal of the American Mosquito*

- Control Association 10: 456–459.
- Lowther, J. K. & D. M. Wood. 1964. Specificity of a black fly, *Simulium euryadminiculum* Davies, toward its host, the common loon. Canadian Entomologist 96: 911–913.
- Madahar, D. P. 1969. The salivary gland chromosomes of seven taxa in the subgenus *Stegopterna* (Diptera, Simuliidae, *Cnephia*). Canadian Journal of Zoology 47: 115–119.
- Moulton, J. K. 2003. Can the current molecular arsenal adequately track rapid divergence events within Simuliidae (Diptera)? Molecular Phylogeny and Evolution 27: 43–57.
- Ottonen, P. O. 1966. The salivary gland chromosomes of six species in the IIS-1 group of *Prosimulium* Roub. (Diptera: Simuliidae). Canadian Journal of Zoology 44: 677–701.
- Peterson, B. V. 1970. The *Prosimulium* of Canada and Alaska. Memoirs of the Entomological Society of Canada 69: 1–216.
- Rubtsov, I. A. 1956. Blackflies (fam. Simuliidae) [Moshki (sem. Simuliidae)]. Fauna of the USSR. New Series No. 64, Insects, Diptera 6 (6). Akademii Nauk SSSR, Moscow & Leningrad [= St. Petersburg], Russia. 859 pp. [In Russian; English translation: 1990. Blackflies (Simuliidae). Second Edition. Fauna of the USSR. Diptera, 6 (6). E. J. Brill, Leiden. 1042 pp].
- Wood, D. M. 1959. Studies on the beetles *Leptinillus validus* (Horn) and *Platypsyllus castoris* Ritsema (Coleoptera: Leptinidae) from beaver. M. A. thesis. University of Toronto, Toronto, Ontario.
- Wood, D. M. 1963a. An interpretation of the phylogeny of the *Eusimulium*-group (Diptera: Simuliidae) with descriptions of six new species. Ph. D. thesis. McMaster University, Hamilton, Ontario. 234 pp.
- Wood, D. M. 1963b. Two new species of Ontario black flies (Diptera: Simuliidae). Proceedings of the Entomological Society of Ontario 93: 94–98.
- Wood, D. M. & D. M. Davies. 1966. Some methods of rearing and collecting black flies (Diptera: Simuliidae). Proceedings of the Entomological Society of Ontario 96: 81–90.
- Wood, D. M., P. T. Dang & R. A. Ellis. 1979. The mosquitoes of Canada (Diptera: Culicidae). Insects and Arachnids of Canada, Part 6. Agriculture Canada Publication 1686: 1–390 + 1 table.

### **Publications on the Simuliidae by D. M. Wood**

- Adler, P. H. & D. M. Wood. 1991. A new North American species of *Simulium* (*Hellichiella*) (Diptera: Simuliidae). Canadian Journal of Zoology 69: 2867–2872.
- Adler, P. H., D. C. Currie & D. M. Wood. 2004. *The Black Flies (Simuliidae) of North America*. Cornell University Press, Ithaca, NY. xv + 941 pp. + 24 color plates.
- Borkent, A. & D. M. Wood. 1986. The first and second larval instars and the egg of *Parasimulium stonei* Peterson (Diptera: Simuliidae). Proceedings of the Entomological Society of Washington 88: 287–296.
- Brown, B. V., A. Borkent, J. M. Cumming, D. M. Wood, N. E. Woodley & M. A. Zumbado (eds.) 2009. *Manual of Central American Diptera*. Volume 1. National Research Council Press, Ottawa, Ontario, Canada. 714 pp.
- Davies, D. M., B. V. Peterson & D. M. Wood. 1962. The black flies (Diptera: Simuliidae) of Ontario. Part I. Adult identification and distribution with descriptions of six new species. Proceedings of the Entomological Society of Ontario 92: 70–154.

- Fallis, A. M. and D. M. Wood. 1957. Biting midges (Diptera: Ceratopogonidae) as intermediate hosts for *Haemoproteus* of ducks. *Canadian Journal of Zoology* 34: 425-435.
- Hunter, F. F., S. G. Burgin & D. M. Wood. 1994. New techniques for rearing black flies from pupae (Diptera: Simuliidae). *Journal of the American Mosquito Control Association* 10: 456-459.
- Laird, M., A. Aubin, P. Belton, M. M. Chance, F. J. H. Fredeen, W. O. Haufe, H. B. N. Hynes, D. J. Lewis, I. S. Lindsay, D. M. McLean, G. A. Surgeoner, D. M. Wood & M. D. Sutton. 1982. Biting flies in Canada: health effects and economic consequences. National Research Council of Canada Publication No. 19248: 1-157.
- Lowther, J. K. & D. M. Wood. 1964. Specificity of a black fly, *Simulium euryadminiculum* Davies, toward its host, the common loon. *Canadian Entomologist* 96: 911-913.
- McAlpine, J. F., B. V. Peterson, G. E. Shewell, H. J. Teskey, J. R. Vockeroth & D. M. Wood (eds.). 1981. *Manual of Nearctic Diptera*. Volume 1. Monograph No. 27. Research Branch, Agriculture Canada, Ottawa. 674 pp.
- Wood, D. M. 1963a. An interpretation of the phylogeny of the *Eusimulium*-group (Diptera: Simuliidae) with descriptions of six new species. Ph. D. thesis. McMaster University, Hamilton, Ontario. 234 pp.
- Wood, D. M. 1963b. Two new species of Ontario black flies (Diptera: Simuliidae). *Proceedings of the Entomological Society of Ontario* 93: 94-98.
- Wood, D. M. 1978. Taxonomy of the Nearctic species of *Twinnia* and *Gymnopais* (Diptera: Simuliidae) and a discussion of the ancestry of the Simuliidae. *Canadian Entomologist* 110: 1297-1337.
- Wood, D. M. 1985. Biting flies attacking man and livestock in Canada. Agriculture Canada Publication 1781/E. 38 pp.
- Wood, D. M. 1991. Homology and phylogenetic implications of male genitalia in Diptera. The ground plan. Pp. 255-284. *In* L. Weismann, I. Országh & A. C. Pont (eds). *Proceedings of the Second International Congress of Dipterology*. SPB Academic Publishing by The Hague, The Netherlands. 367 pp.
- Wood, D. M. & A. Borkent. 1982. Description of the female of *Parasimulium crosskeyi* Peterson (Diptera: Simuliidae), and a discussion of the phylogenetic position of the genus. *Memoirs of the Entomological Society of Washington* 10: 193-210.
- Wood, D. M. & A. Borkent. 1989. Phylogeny and classification of the Nematocera. Pp. 1333-1370. *In* J. F. McAlpine (ed.), *Manual of Nearctic Diptera*. Volume 3. Monograph No. 32. Research Branch, Agriculture Canada, Ottawa. 249 pp. [pp. 1333-1581].
- Wood, D. M. & D. M. Davies. 1965. The rearing of simuliids (Diptera). *Proceedings of the Twelfth International Congress of Entomology, London (1964)*: 821-823.
- Wood, D. M. & D. M. Davies. 1966. Some methods of rearing and collecting black flies (Diptera: Simuliidae). *Proceedings of the Entomological Society of Ontario* 96: 81-90.
- Wood, D. M., B. V. Peterson, D. M. Davies & H. Györkos. 1963. The black flies (Diptera: Simuliidae) of Ontario. Part II. Larval identification, with descriptions and illustrations. *Proceedings of the Entomological Society of Ontario* 93: 99-129.

## SCIENTIFIC PAPERS

### **Intriguing Genes: Expressed Sequences from the *Simulium vittatum-tribulatum* complex. II. CO<sub>2</sub> Related Gene Expression.**

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#### **Introduction:**

We continue our presentation of biologically interesting expressed gene sequences from *Simulium vittatum* adults and *S. tribulatum* larvae, focusing on CO<sub>2</sub>-related sequences in this issue. CO<sub>2</sub> is long established as an important attractant for blood-feeding arthropods, including simuliids (e.g., Sutcliffe et al., 1995; Burkett-Cadena et al., 2015), and so we anticipate interest from the community in this gene set. The molecular mechanism of CO<sub>2</sub> detection is well studied in model-system dipterans (e.g., Jones et al., 2007), and several different GO terms are applicable. These sequences were retrieved from our annotated transcriptomes using standard Gene Ontology expressions (<http://geneontology.org>): "detection of carbon dioxide", "response to carbon dioxide", and "cellular response to carbon dioxide".

To reiterate, we intend these sequences as starting points for further biological investigations in the Simuliidae community. We are happy to consider any specific GO term requests for upcoming issues of the Bulletin. Please contact the corresponding author (CLB) if your work would be helped by *S. vittatum-tribulatum* sequences.

#### **Results:**

Each sequence is named with the in-house designation of the *S. vittatum-tribulatum* database; GenBank accession numbers will be provided as a supplement prior to publication of the full database.

Of 20 sequences examined, all corresponding protein matches fell into one of the following categories: gustatory and odorant receptor (1), carbonic anhydrase (2; involved in cellular CO<sub>2</sub> physiology), or guanine and nucleotide-binding protein (17; signal transduction). Note that most of these sequences are also annotated with other GO terms, and any one sequence might not serve that specific function in the stage expressed. Five sequences representing the groups are featured with their mRNA sequences, protein sequences, and effective FPKM values (a standard measure of expression level: fragments per thousand bases of sequence per million mapped reads) for the two species and various stages of development.

### **GO category: "detection of carbon dioxide" (3031)**

**Sequence name:** c120728\_g1\_i1|m.5402

**Protein category:** Gustatory and odorant receptor

**mRNA sequence:**

>c120728\_g1\_i1|m.5402

```

GCACTTTTGGATCCCCTTTGTCGGCTGGGGTGTAGCCGTCGACGTGGCCGTTTA
CAAGACCATGTGGGGCTCCTTTCAAGTGCGGTACTTTTCGTGTAACCTGGCGAATC
CTTGAAGTTCCCAACCTCAAATCCTGATCGTGCTCTTTTCGATCGGATGTTTG
GTTTGCGCCATTCTCTTCTTGATATCGTTGAGCTTGCTGCTCGAAGGGTTTTTCGT
TGTGGCATAACGACGGCCTATTACCACATCATCACGATGTTGAACATGAATTCGGC
GTTGTGGTACATCAACAGCCGGGGCATTTCGTGTGGCGTCAACAGTTTGGCCA
AGTGTTCGCAAAGATGTTCGGCATCGAGTGTACGGCCGCACTTATCTCGCAGT
ACCGGTTCTTGTGGCTGAACTTGAGCGAGATGTTGCAGGCGCTGGGCAATGCG
TACGCGCTACCTACTCAACCTATTGCTTGTTTATGTGAGTTTTGCATGCATTTCT
TAGTTGTTAATAAAAAATATGTCTAACTCCCAGGTTTCGTCAACATCACGATCGCA
ATCTACGGTGCCTCTCCGAAATCA

```

**Protein sequence:**

>c120728\_g1\_i1|m.5402

```

HFWIPVGVGVAVDVAVYKTMWGSFQVRYFRVTGESLKFNPILKILIVLFSIGCLV
CAILFLISLSLLLEGFSLWHTTAYYHIITMLNMNSALWYINSRGIRVASNSLAKCFR
KDVGIECTAALISQYRFLWLNLSEMLQALGNAYARTYSTYCLFM

```

**Effective FPKM:**

Vittatum adult male: 0

Vittatum adult nulliparous female: 3.47

Vittatum adult parous female: 0

Tribulatum female larvae: 0

Tribulatum male larvae: 0

This gene is the only sequence retrieved by the "Detection of carbon dioxide" GO term (3031). It is particularly interesting because of its expression pattern, and homology to known *Anopheles gambiae* and *Drosophila* genes (GPRgr22 and Gr21a, respectively; Jones et al., 2007). Expression is restricted to nulliparous females (Figure 1). Even though S.

*vittatum* is primiparously autogenous, the expression pattern suggests a relation to prey-location, which Sutcliffe et al., (1995) showed is highly CO<sub>2</sub> dependent in *Simulium arcticum*.

In *Anopheles* CO<sub>2</sub> detection depends on co-expression of GPRgr22 and GPRgr24 (Jones et al, 2007). We have not detected a GPRgr24 homologue, but this could be due to simple sampling error, the primiparous autogeny noted above, or a real difference in the biology of CO<sub>2</sub> detection between the two families.



**GO category: "response to carbon dioxide" (10037)**

**Sequence name:** c159754\_g1\_i1|m.46542

**Protein category:** Carbonic anhydrase

**mRNA sequence:**

>c120728\_g1\_i1|m.5402

```

GCAC TTTTGGATCCCCTTTGTGCGGCTGGGGTGTAGCCGTCGACGTGGCCGTTTA
CAAGACCATGTGGGGCTCCTTTCAAGTGCGGTACTTTTCGTGTAAC TGGCGAATC
CTTGAAGTTCCCCAACCTCAAATCCTGATCGTGCTCTTTTCGATCGGATGTTTG
GTTTGCGCCATTCTTTCTTGATATCGTTGAGCTTGCTGCTCGAAGGGTTTTTCGT
TGTGGCATACGACGGCCTATTACCACATCATCACGATGTTGAACATGAATTCGGC
GTTGTGGTACATCAACAGCCGGGGCATTTCGTGTGGCGTCCAACAGTTTGGCCA
AGTGTTCGCAAAGATGTCGGCATCGAGTGTACGGCCGCACTTATCTCGCAGT
ACCGGTTCTTGTGGCTGAACTTGAGCGAGATGTTGCAGGCGCTGGGCAATGCG

```

TACGCGCGTACCTACTCAACCTATTGCTTGTTTATGTGAGTTTTGCATGCATTTCT  
TAGTTGTTAATAAAAAATATGTCTAACTCCCAGGTTCTGCAACATCACGATCGCA  
ATCTACGGTGCACCTCCGAAATCA

**Protein sequence:**

HFWIPVGVGWAVDVAVYKTMWGSFQVRYFRVTGESLKFPNLKILIVLFSIGCLV  
CAILFLISLSLLEGFSLWHTTAYYHIITMLNMNSALWYINSRGIRVASNSLAKCFR  
KDVGIECTAALISQYRFLWLNLSEMLQALGNAYARTYSTYCLFM

**Effective FPKM:**

Vittatum adult male: 22.04  
Vittatum adult nulliparous female: 14.51  
Vittatum adult parous female: 20.28  
Tribulatum female larvae: 22.78  
Tribulatum male larvae: 24.01

**GO Category: "response to carbon dioxide" (10037)**

**Sequence name:** c159754\_g1\_i2|m.46543

**Protein category:** Carbonic anhydrase

**mRNA sequence:**

>c159754\_g1\_i2|m.46543

AGAAAATAACACACATCAATACTTTCCATCTCAAAAATTCTCAAAAATCTTTAAAA  
AACGTTAAAAAAATCTAGAAAACTCCTGAAAACTCTAATTATGGTGTGGCCAC  
CAAGTAACAAAATGAGCTTTTTGCAACAAGCCGGCATTGACACAAAAGTCAGGTT  
ATCAAAAAATCGGTGACCAAAGTCCAACGAAAAGTGCTCTCGTCCAGAGAAT  
GCATTTTTCGAAGCAGTTTTCCCATCGGCCAGCATTCCCATTGACCCCGAACCCAG  
TTGTCACCCTTTTCTCAATATTTCAAGTGGTCTGTAATCAGTTTTCGAAAAGAAATT  
GCATAATGGAGAAAAATTTACGAGGAATTATGAGGTATCGCAACACGACACGCG  
ATGTGATGGTCAAGGAGTTCAAGCAGGTCAAAGATAATCCAAAGCCCAAAGCC  
GTCTTCTTACATGCATGGATTCAAGGATGTTGCCCACTCGGTTACCCGAAACA  
CATGTGGGTGACATGTTTCATTCGTAATGCCGGAACCTGATACCTCATGCC  
GAACACTTCCAAGATGAGTATTATAGTTGTGAACCGGCCGGACTGGAGCTGGG  
CTGTGTGGTCAACGATATCAGGCACATTGTTGTGTGCGGTACAGTGACTGTAA  
GGCCATGAATCTACTGCACCAAGTTACAGGACCCCGTAATGGCGTCAGAGGTGA  
GTACTTACCGACCTAGGGAGTTCCAAAATCGGTATAAGCTTTAAAATAAAAAAAA  
ATCACTTTAGGATAATCGTCGCATATCACCTTACGTGCCTGGCTGTGCACCCAC  
GCCACACCAGTTTGGAAAATTCAAGCATCTCGAAGAAGTGGGTTTTGACAAG  
CCACTGACATTTTCATCGGAACTCCAATGCGGAAATTTGTCGCCTACATTGATC  
CAGACAATCAATTTTGCATCGAAGACAAAAGTGTCTCAGATCAACACACTACAGC  
AGATGCAAAATGTGGCAAGCTATGGATTTCTGAAGAGTCGCCTGGAGCGACAC  
GACTTGACATATCCACGCGCTCTGGTTCGATATCTATACTGGTGATATTTATTA  
TAGTCGCGGTTCCAAACGATTTGTGCCCATCGATGAAGGAAACGTTGAGGACCT  
GGTGAAGAAGTGCGAAAGTACTATTCGTGAGTTGAGAAAAAGTGCCTTTGAA  
CTTGGTGTGTGCGTGGGTTTTTTAGGTGAATTTGTTTGACGACATTATGACCTT  
TCTGTTTTGGTTGGGTTACCCGAAATTCGTGCGCAAATTAGGTTAGGATTTTTGA

TATGATTTTTATTAGCCTTAGAAACTTATCATTCCATACAACAATACGAGTATAC  
 ATTGCATGGCTTAAGTCTAAAGTATATATACATAATATGCCAATTTATCCCCTGT  
 TGGAGTGACTCAAATATTCACATAAAAACTTTGATAAACTATATTTATTGACCAA  
 AATCCAAATTTAGATATTTATATCTACATATTAATACTTGCATTTATTTTATGAAA  
 ACAATGTTATATTATAATGATTAATCTATATTTACTGTTGACAATCCCCGATAATTAT  
 GATGAAATCTATATTTTTAAACAATGGCGACCATCAACCATGTACTTATATTTAA  
 GTA AACACGAAATTGTTTATTTTCTGAAGAATTTTATGTAGATAGATGCGAAAA

**Protein sequence:**

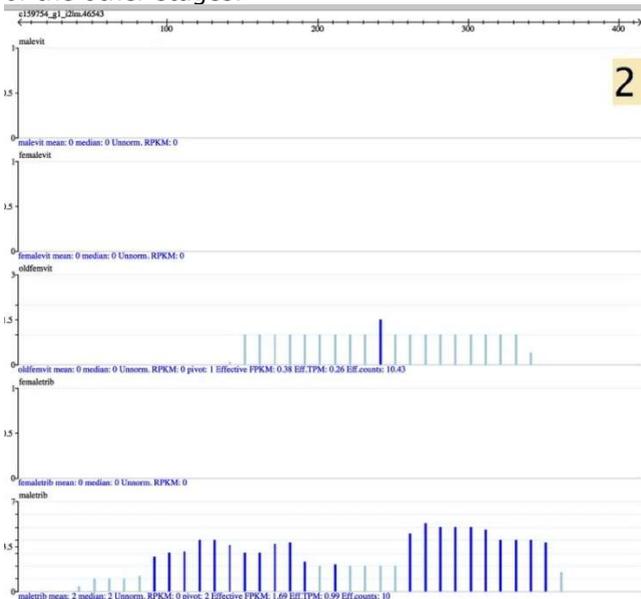
>c159754\_g1\_i2|m.46543

MEKILRGIMRYRNTTRDVMVKEFKQVKDNPKP KAVFFTCMDSRMLPTRFTETHVG  
 DMFIIRNAGNLIPHAHFQDEYYSCEPAGLELGCVVNDIRHIVVCGHSDCKAMNL  
 LHQLQDPMASEVSTYRPREFQNRKYL

**Effective FPKM:**

Vittatum adult male: 0  
 Vittatum adult nulliparous female: 0  
 Vittatum adult parous female: 0.38  
 Tribulatum female larvae: 0  
 Tribulatum male larvae: 1.69

This sequence was included from among several other carbonic anhydrases due to its unusual expression pattern: a very low level in male larvae and parous females (Figure 2). Other carbonic anhydrases had more uniform expression patterns (e.g., c159754\_g1\_i1|m.46542 above). It is possible low expression levels were missed by chance in some or all of the other stages.



**GO Category: "cellular response to carbon dioxide" (71244)**

**Sequence name:** c148968\_g1\_i1|m.16506

**Protein category:** Guanine nucleotide-binding protein (G protein)

**mRNA sequence:**

>c148968\_g1\_i1|m.16506

CAGATATAAAAAAAAAATTGGTCGGGACAATTTTTATATTTTTTAAAAATTCAGGAA  
AAAAGTAAATTACAAACCAAAAGCAAAAGTTTCGGTAGTTGCTAAAAGATTACACA  
ATCGGCCCTCCGTTCTGGCCGATAGTGCGGCACTCGACAGTTAAACAAGAATGAA  
AATTAAGAAAAAGATCAAAGTAACTGCGCAACGGTGAAGTGACTAATTTTTACAC  
ATAAGACACGCAGTGGTAAATGACATTCAAAGATTTTTATACCAAAAACCTTAGTT  
AGAAAAATATTGTGCTTGCTAAACATTGTAGAAAAGCTACAAAGGTCTGTTGGACC  
CTGCCTTGGATTGTAATCGGATAGTGTAGTGTGGTAGGCAGGTTTCAGAGAGCA  
TTGTTGCCAGTGAATTTTTGATAAGCTATATCTAATATACAAATTAATGAAAAATTA  
AAACCGGAAATAAAATTGGAAAAAGGATAACATTGTTGTGAGAGATTGTAACAAA  
ATTAACAGTACAAAAATGATGGAGTGCTGCTTGTCCGAGGAGGCGAAGGAACA  
AAAACGCATCAATCAGGAAATTGAGCGGCAACTTCGCCGGGATAAAAGGGATG  
CACGACGGGAGCTGAAGTTGCTATTGCTTGGCACTGGTGAATCTGGAAAGTCTA  
CATTCAACAAATGAGAATTATTCATGGTGCCGGCTATTCAGACGAGGATAA  
GCGTGGTTTCATCAAACCTGGTCTACCAAAAATTTTTCATGGCCATGCAATCCATG  
ATTCGTGCCATGGATCTATTGAAAATAGTTTATATTAATCCCAGCTGTAATACACA  
CGCTGAACTTATAAAGGAGGTGGATTTTAAAACGGTAACATCGTTCGAGGAACC  
ATATGTCAAAGCAATAAAAGATTTGTGGGCAGATGGTGGTATCCAGGAGTGCTA  
TGACCGAAGAAGAGAATATCAGCTAACAGATTCAGCAAAATACTACTTAATGGAA  
ATCGACCGAGTGGCCGCCACCAACTACCTACCAACGGAACAAGATTTTTGAGG  
GTTTCGAGTACCAACAACCTGGAATAATTGAATATCCATTTGATTTGGAAGAGATT  
GATTTAGAATGGTGGAGCTAGGTTGGTCAGAGATCTGAGCGAAGGAAATGGATC  
CATTGTTTTGAGAATGTAACATCGATTATATTCTTAGTAGCGCTGTCCGAATATGA  
TCAAATTTTGTGAGTCAGAAAACGAGAATCGTATGGAAAGAGTCCAAAGCATT  
TTCAAACCATTATCACATATCCATGGTTCCAACATTCATCAGTTATTCTCTTCTTG  
AACAGAAAAGATTTGTTAGAAGAAAAGATAATGTATTACATTTGGTAGACTATTT  
TCCAGAATACGATGGACCACAACGGGATGCAATAACAGCCAGAGAATTTATTCT  
TAGAATGTTTGTAGATTTAAATCCAGATTCGGAAAAAATAATTTATTACATTTAC  
ATGTGCGACAGATACGAAAAACATTTCGATTCGTGTTTCGAGCCGTCAAAGATAC  
AATTCCTCAATCTAACTTGAAAAGAGTATAACTTAGTCTGAATAGGAATTGAAACT  
GGATTGTTGATCTTTTTCTATATAATTAACAAAAATAAAAAACCCATTTGAAC  
GCAACATTTACACAAGTCGCAGCAGTAACCAATAAAATGCAAGCAACTTCCA  
ACATGAGAAAAATGACGACGAACATTACACAATCCAGTTGAATATCGACAAAAATG  
CATTTCAATATGTGCAATATATAAATTTTCATAAACCAAAATCAAAAAAGATAAAGA  
AAATTAACAAGCAAAAAACAAGCATTTCGCTTGGCATTTCGAAAAAGTTCTCAAATTG  
ACGAAAAACGGAACAAGTCACTTATAAAATTTTGTGTTAATTTTTTTTTAAATTA  
AAGAGGAAAAACA AAAAATTCAAACAAAGGGAAATTTCAAAGCTAAACGATA  
CATTAAAATAAACATAGAATTGAAACGCTGATGTCGATATGTGTAATTATAAATA  
AGTTATGAAGAAACAGAACTATCGTAAGAAATAATGAAAATATAAAAAATACATCG  
TTCGCACAATTTTTGTCCGCCAGTTTTAGAAAAAAAACAGAAAAACAACAACA

ACACATAAAGAAATAAAAAAAAAAGGAAAAATCTTTACGTAAAAACACATCATAATAAT  
ATAAAGTTTGTATATTTTTATTATTTTACACTCAGAAAAGTCAAAAACAAAACATTT  
GTATTTTATAGTGAACGTTCTATCACACAGACCCAAGCATAAATTCACTCAGATT  
ATACAAAGGAACACAAATCATAGCCGAGTAACACAAAACGAAATACATAAACAC  
GCATAAATACATAAAATGATTACAAAATGTGTAACCTGATCCATTAGAAGATAAA  
ATCAATTTGTTGTTCTATTTTGTGATTCTATTGATTTGGCATTTCATAGCCT  
GATCCCCGTTTTGCGCTACAATCGATGTAATCCGACATTTTTTCGATAAGCAGC  
GCACCGTTTTCAATTATATAACGTTCAATAATTAAGTCTGGTAGACAAAACCGAAA  
GGAATAACAGCCGCGCGTTTCGATAATTTTTGTACAAAACGAAAAATTAATTCAA  
TATTTTACTATATATAATTATATATTTTTGGATGTTGTTTTGATTTTCATAATCAGC  
ATAATAAACTTATATTACTATTCCCTAATTAATAATATTATGTATGTTTCATCAAT  
TTTTCCATAATTTCTATGCTGTTTTAATTTAAAAAATTATTATTTTTCTAGAAAC  
AACTAAATGAACCTAAGAAGTAGAATACCCGTCGACTCTTTATAACGTGATCTCTTT  
AGCGTCGATTTTTGTTATTTTTCCAGTGTGTTTTCCGTTTCATCAATTTTTTCT  
CGTGTATCGCCTTGCAACTGAGCATTACTTATTTAAGGGCTTTCAATGTTATGCA  
ATAAAAATTATAATGTTATGGGAAGATACAGAAAACCAACGCACAAAACTTTACA  
CAGCAGTCATTTCAAGATTTATTTATAAAGAGTTGACTGCATTGCGGGATATTA  
AAGTTGAAATGATAAAAAAAATTGTTTCACTTTTTTATCCAAAACATTTAAAGAA  
ATATTTATTAATGTTTTATCTATTTTATGTGACCTCACTAAAAAACCATGAAA  
GAACTTGCAACTAGAAAAGTCCTTGATAATTTGCATGTGAAATTCATTATAATTTA  
AAAAGTGTGCAAAGCATTGAACATATTTTTTTTTGTAATTGTTATTTTTTATATCAC  
AATATTCACATATAATAATTATTTAAAGCGATTATTTAAATAAAGTATGTTATACGAC  
TTGTTTTATTTTTATTTTTATTGGTTTTTTGTTTAAATTTTTATTTCGATTTTTAATTTAT  
TTATTCTTAAACTTCGCAATGACTTTTTTGAATTTTGCGGCAACCCGTTTTGA  
ACATAGGGTGGAGCGGTGCCTGTTACTTTGAAGCCGTTTCGATGCCATTATC  
GGTTTAGAATAAGATAAATGTGAAAATGCGGGAGATCTTCAACAATTTAAGTGGT  
ACAATTTAGTCATTTTGCTTTGATTACCGATCAAAGTAACTGTAAAGTTTCATTCC  
GTTTTTGCAGTTAAAAATATTTTTAAAAAACCTTACAACCAATATCTAACATTGTG  
CACTCACTCCGAAGGGGAAAAATATAAATGAAAAATAAACAACAATTGAAATG  
ATGTATGAATTGAAATACTGAAATAAACATAAAAAATGAAAAAAAAGAAAACCTT

**Protein sequence:**

>c148968\_g1\_i1|m.16506

MMECCLEEEAKEQKRINQEIERQLRRDKRDARRELKLLLLGTGESGKSTFIKQMR  
IIHGAGYSDKDRGFIKLVYQNIQFAMQSMIRAMDLLKIVYINPDCNTHAEIKV  
DFETVTSFEPEYKAIKDLWADGGIQECYDRRREYQLTDSAKYYLMEIDRVAATNY  
LPTEQDILRVVPTTGIIIEYFPDLEEIRFRMVDPVGGQRSERRKWIHCFENVTSIIFL  
ValseyDQILFESENEENRMEESKALFKTIITYPWFQHSSVILFLNKKDLLEEKIMYS  
HLVDYFPYDGPQRDAITAREFILRMFVDLNPDESEKIIYSHFTCATDTENIRVFVAA  
VKDITLQSNLKEYNLV

**Effective FPKM:**

- Vittatum adult male: 82.07
- Vittatum adult nulliparous female: 89
- Vittatum adult parous female: 44.76
- Tribulatum female larvae: 32.38
- Tribulatum male larvae: 41.88

G proteins are involved in signal transduction; transmitting messages from outside the cell to initiate responses. This class of protein was discovered accidentally by Rodbell's lab due to a novel activity picked up when using commercial ATP preparations contaminated with GTP as opposed to their own in-house synthesized ATP (<http://www.nobelprize.org>. Physiology or Medicine 1994).

### **Conclusion:**

We anticipate submission of the full database in summer of 2021 but are happy to provide sequences of interest prior to that.

### **Literature Cited:**

- Burkett-Cadena, N.D., Blosser, E.M., Young, R.M., Toé, L.D. & T. R. Unnasch 2015. Carbon dioxide generated from carbonates and acids for sampling blood-feeding arthropods. *Acta Trop.* 2015 Sep;149:254-61. doi: 10.1016/j.actatropica. .06.019. Epub 2015 Jun 20. PMID: 26103427.
- Jones, W.D., Cayirlioglu, P., Grunwald Kadow, I. & L.B. Vosshall. 2007. Two chemosensory receptors together mediate carbon dioxide detection in *Drosophila*. *Nature* 445:86-90(2007)
- Sutcliffe, J., Steer, D., & D. Beardsall. 1995. Studies of host location behaviour in the black fly *Simulium arcticum* (IIS-10.11) (Diptera: Simuliidae): Aspects of close range trap orientation. *Bulletin of Entomological Research*, 85(3), 415-424. doi:10.1017/S0007485300036142



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