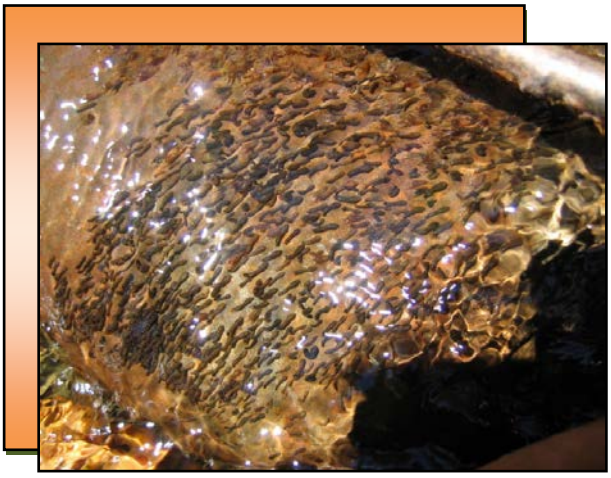
North American Black Fly Association

10th Annual Meeting



February 8th – 10th, 2012

Program Agenda

Archbold Biological Station • Venus, Florida

SCHEDULE OF PRESENTATIONS AND EVENTS

WEDNESDAY, FEBRUARY 8TH

2:00 PM – 2:25 PM	WELCOME TO ARCHBOLD BIOLOGICAL STATION: MEMBER UPDATES, NABFA WEBSITE, AND TREASURER UPDATE **John Walz**, Metropolitan Mosquito Control District, St. Paul, MN
2:25 PM – 2:50 PM	ANOTHER REMNANT SITE FOR THE ANCESTOR OF *S. SAXOSUM* AND *S. ARCTICUM* S.S. **Gerald F. Shields**, Department of Biological Sciences, Carroll College, Helena, MT
2:50 PM – 3:15 PM	DEFINING THE CYTOTYPE AND PERSISTENCE CONCEPTS IN BLACK FLY CYTOGENETICS Jeanna Van Hoey and **Gerald F. Shields**, Department of Biological Sciences, Carroll College, Helena, MT
3:15 PM – 3:45 PM	**BREAK**
3:45 PM – 4:10 PM	WHY DO TRICHOMYCETES PREFER BLACK FLY MIDGUTS TO MOSQUITO MIDGUTS? **Charles E. Beard**[1] and Abdullah Inci[2] [1]Clemson University, Clemson, SC [2]Erciyes University, Turkey
4:10 PM – 4:35 PM	TWELVE YEARS OF CYTOGENETIC ANALYSIS OF THE *S. ARCTICUM* COMPLEX IN WESTERN MONTANA; CORRELATIONS WITH ENVIRONMENTAL AND BIOLOGICAL FACTORS **Gerald F. Shields**, Department of Biological Sciences, Carroll College, Helena, MT
4:35 PM – 5:00 PM	METROPOLITAN MOSQUITO CONTROL DISTRICT (MMCD) BLACK FLY PROGRAM UPDATE WITH NATIONAL POLLUTANT DISCHARGE ELIMINATION SYSTEM (NPDES) PERMIT UPDATE **John Walz**, Metropolitan Mosquito Control District, St. Paul, MN
5:00 PM – 6:00 PM	**SOCIAL HOUR**
6:00 PM	**DINNER**

THURSDAY, FEBRUARY 9TH

6:00 AM – 8:30 AM	CONTINENTAL BREAKFAST IN DINING ROOM

THURSDAY, FEBRUARY 9TH (CONTINUED)

9:00 AM – 9:25 AM

ASSESSING REPRODUCTIVE ISOLATION AMONG CRYPTIC BLACK FLY SPECIES

Ida M. Conflitti[1,2], Gerald F. Shields[3], Robert W. Murphy[1,2], and Douglas C. Currie[1,2]
[1]Department of Ecology and Evolutionary Biology, University of Toronto, Toronto, Ontario, Canada
[2]Department of Natural History, Royal Ontario Museum, Toronto, Ontario, Canada
[3]Department of Natural Sciences, Carroll College, Helena, Montana, USA

9:25 AM – 9:50 AM

A SIDE ORDER OF FLIES: UPDATE ON THE BITING FLY COMPONENT OF THE NORTHERN BIODIVERSITY PROGRAM

Patrick Schaefer[1,2] and Douglas C. Currie[1,2]
[1]Ecology and Evolutionary Biology, University of Toronto, Toronto, ON, Canada
[2]Department of Natural History, Royal Ontario Museum, Toronto, ON, Canada

9:50 AM – 10:15 AM

EXPANDED SURVEYS AND NEW SPECIES OF BLACK FLY GUT FUNGI IN SOUTHERN IDAHO

Nicole Reynolds, Emma Wilson, Prasanna Kandel, Matthew Laramie and Merlin White, Boise State University, Department of Biological Sciences, Boise, ID

10:15 AM – 10:45 AM

BREAK

10:45 AM – 11:10 AM

NON-TARGET EFFECTS OF FUNGICIDES ON BLACK FLY ASSOCIATED GUT FUNGI AND ACCUMULATION OF PESTICIDES IN LARVAL TISSUE

Emma R. Wilson[1], Kelly L. Smalling[2], Timothy J. Reilly[3], Lance Steele[1], Prasanna Kandel[1], Alison B. Chamberlin[1], Justin W. Gause[1], and Merlin M. White[1]
[1]Boise State University, Department of Biological Sciences, Boise, ID
[2]US Geological Survey, California Water Science Center, Sacramento, CA
[3]US Geological Survey, New Jersey Water Science Center, West Trenton, NJ

11:10 AM – 11:35 AM

DEVELOPMENT OF AN AREA-SPECIFIC PICTORIAL KEY FOR STREAMLINED LARVAL BLACK FLY MONITORING EFFORTS

Kristin E. Sloyer and John R. Wallace, Millersville University, Millersville, PA

11:35 AM – 12:00 PM

GRADUATE STUDENT RESEARCH AT UGA: FOCUS ON OVIPOSTION BEHAVIOR AND LARVAL FEEDING

Elmer W. Gray, Department of Entomology, University of Georgia, Athens, GA

12:00 PM – 1:30 PM

LUNCH

1:30 PM – 1:55 PM

DNA BARCODING THE BLACK FLY GENUS *CNEPHIA*

Kenneth Pruess, University of Nebraska, Lincoln, NE

THURSDAY, FEBRUARY 9TH (CONTINUED)

1:55 PM – 2:35 PM	BLACK FLIES AND THE CRITICALLY ENDANGERED WHOOPING CRANE: IS THERE A LINK? **Elmer W. Gray**[1], Peter Adler[2], and John Smink [1]Department of Entomology, University of Georgia, Athens, GA [2]Clemson University, Clemson, SC
2:35 PM – 3:00 PM	**PRESENTATION OF *MIKE SPIRONELLO AWARD* FOR OUTSTANDING STUDENT PRESENTATION**
3:00 PM – 3:30 PM	**BREAK**
3:30 PM – 4:30 PM	**GENERAL DISCUSSION / BUSINESS MEETING**
4:30 PM – 5:00 PM	**GROUP PICTURE**
5:00 PM – 6:00 PM	**SOCIAL HOUR**
6:00 PM	**BBQ DINNER**

FRIDAY, FEBRUARY 10TH

6:00 AM – 8:30 AM	CONTINENTAL BREAKFAST IN DINING ROOM
ANYTIME	NATURE TRAIL TOUR OF ARCHBOLD BIOLOGICAL STATION (OPTIONAL) *(self-guided ½ mile walk through the scrub, trail booklets available at main office)*

Thanks to Archbold Biological Station /www.archbold-station.org/ for allowing NABFA to host our meeting at their facility.

Program editor: Carey LaMere

NABFA WEBSITE: /www.nabfa-blackfly.org/

10TH ANNUAL NABFA MEETING
PRESENTATIONS TITLES WITH ABSTRACTS

FEBRUARY 8 – 10, 2012
ARCHBOLD BIOLOGICAL STATION
VENUS, FLORIDA
(In alphabetical order by presenter)

WHY DO TRICHOMYCETES PREFER BLACK FLY MIDGUTS TO MOSQUITO MIDGUTS?

Charles E. Beard[1] and Abdullah Inci[2]
[1]Clemson University, Clemson, SC
[2]Erciyes University, Turkey

Trichomycetes are a group of symbiotic fungi that occupy the midguts or hindguts of several aquatic dipteran families, and especially Simuliidae. However, the similar mosquito midgut does not appear to host these symbiotes. Trichomycete hosts are most commonly aquatic filter-feeding or detritus-grazing Diptera larvae. Observing trichomycetes generally depends on locating live colonized animals and dissecting out the guts. In general, we assume the physiology and gut structure of Blephariceridae, Ceratopogonidae, Chironomidae, Culicidae, Dixidae, Simuliidae, and Thaumaleidae are similar. Because the midguts and hindguts of the hosts are putatively alike we expect trichomycetes to colonize the host families. This is generally the case with the trichomycetes of the hindgut including mosquitoes. However, the midgut fungi have an unexpected distribution. We have never found midgut trichomycetes in mosquitoes, a very common dipteran family. Why are there no midgut trichomycetes in mosquitoes? Is it a behavioral limitation, ecological limitation, physiological limitation, or a structural limitation? We investigated this by collecting black larvae from field sites and placing them in containers. The trichomycete colonized (confirmed by dissection of the sampled larvae) black fly larvae were allowed to feed and shed frass which contained trichomycete spores. Mosquito larvae were then placed in water with spores from the containers and allowed to feed. The mosquito larvae were then dissected and assayed for midgut trichomycetes. Trichomycete fungi were found growing in the mosquito midguts. Prevalence was up to 67% (two samples). This indicates that normally midgut inhabiting trichomycetes (*Harpella* spp.) from black flies are able to be ingested, germinate, attach, and grow in mosquito midguts. These results indicate that mosquito midguts are potential symbiotic host sites and that the lack of these symbioses in field-collected mosquitoes is not a physiological limitation. Natural mosquito populations might be subject to environmental limitations on mosquito colonization by the midgut trichomycetes.

ASSESSING REPRODUCTIVE ISOLATION AMONG CRYPTIC BLACK FLY SPECIES

Ida M. Conflitti[1,2,*], Gerald F. Shields[3], Robert W. Murphy[1,2], and Douglas C. Currie[1,2]
[1]Department of Ecology and Evolutionary Biology, University of Toronto, Toronto, Ontario, Canada
[2]Department of Natural History, Royal Ontario Museum, Toronto, Ontario, Canada
[3]Department of Natural Sciences, Carroll College, Helena, Montana, USA
[*]Presenting author

Cytological analyses reveal that black fly morphospecies are often composites of chromosomally distinct populations. Whether these entities are considered reproductively isolated depends on their stage of chromosome evolution. Focusing on the diverse *Simulium arcticum* species complex, containing nine 'cytospecies' and twenty one 'cytotypes', we aim to understand the molecular basis of

chromosomally induced speciation. Specifically, we test whether cytologically defined taxa reflect underlying genetic structure by estimating population differentiation, gene flow, and phylogenetic history with a suite of molecular markers. Preliminary results of such studies will be presented and future plans discussed.

DOUG CRAIG PAYING TRIBUTE AT THE GRAVE OF DR. ANDREW SINCLAIR, MESOPOTAMIA, RANGITATA RIVER VALLEY, NEW ZEALAND

Sinclair, a naval medical doctor arrived in New Zealand in October 1841 and was in the Bay of Islands, North Island when the British ships, the Terror and Erebus arrived in November to overwinter for two months before heading off to Antarctica. Where, amongst other things, they discovered the Ross Sea. There is a special connection here for Canadians. Two years later the two ships were in the ill-fated Franklin Expedition.

Sinclair was a keen botanist and collected widely and has numerous New Zealand plants named after him. He is, most likely, the person who collected a large sample (ca. 450) of New Zealand simuliids and sent them to the British Museum, apparently on the Terror and the Erebus. So the glass-topped pill box label indicates. Originally the simuliid was to be described as 'Simulium caecutiens Walker, 1848', but for whatever reasons was not, a *nomen nudem*. Slipping under the radar, so-to-speak, the sample was ignored until now. The first formal designation of a New Zealand simuliid was by Schiner, 1868, and is now known as *Austrosimulium australense*. It is indeed the species collected by Sinclair two decades earlier, but this time was based on two badly damaged specimens from Auckland - probably collected by somebody whacking them!

Sinclair was drowned in the Rangitata River in 1861 while on a geological expedition. He was at the time Colonial Secretary, thence the rather formal grave.

A connection to the present is that Doug is wearing the NABFA T-shirt from 2003 that sports an image of *Simulium rothfelsi* larval head.

Mount Sinclair is in the background and it is blowing a very strong, warm Northwester wind - very pleasant. But, under such conditions it is raining hard in the headwater mountains and, as at the time Sinclair drowned, the river is in raging flood!

GRADUATE STUDENT RESEARCH AT UGA: FOCUS ON OVIPOSTION BEHAVIOR AND LARVAL FEEDING

Elmer W. Gray, Department of Entomology, University of Georgia, Athens, GA

No Abstract Submitted

BLACK FLIES AND THE CRITICALLY ENDANGERED WHOOPING CRANE: IS THERE A LINK?

Elmer W. Gray[1], Peter Adler[2], and John Smink
[1]Department of Entomology, University of Georgia, Athens, GA
[2]Clemson University, Clemson, SC

Reintroduction of the critically endangered Whooping Crane, *Grus americana,* was initiated at Necedah National Wildlife Refuge in Necedah, Wisconsin in 2001. The cranes began attempting to nest at this site in 2005. An undesirable phenomenon of an unusually high incidence of nest desertion has been observed each subsequent year. Significant populations of black flies (Diptera: Simuliidae) have been observed on the nesting birds and around the nest sites. The primary pest species have been identified as *Simulium annulus* and *S. johannseni*. At the request of the U.S. Fish and Wildlife Service

and the Whooping Crane Eastern Partnership, larvicide applications have been conducted with the biological larvicide *Bacillus thuringiensis* var. *israelensis* (*Bti*). Effective larval mortality was observed over 32 miles of river representing the primary larval habitat of the pest species. Water temperatures in this habitat were 1-2 °C. Adult populations of the pest species were significantly reduced on the refuge as compared to the baseline year of 2009. Biologists determined that 6 of 20 first-time nests were incubated to full term in 2011, as opposed to 0 of 43 for the previous six years. This work represents the first time that a correlation has been made between black fly populations and whooping crane nesting success. Furthermore, this is the first time that *Bti* has been used to suppress black fly populations that are attacking an endangered species.

DNA BARCODING THE BLACK FLY GENUS *CNEPHIA*

Kenneth Pruess, University of Nebraska, Lincoln, NE

A portion of the mitochondrial CO-I gene was sequences from 5 species: *Cnephia dacotensis*, *C. pecuarum*, *C. ornithophilia*, *C. eremites*, and *C. pallipes*. *C. ornithophilia* was the most diverse species with greatest variation west of the Mississippi River and included a clade found only in that area. Diversity decreased from south to north. *C. dacotensis* had limited variation but did form two clades, one northern, the other southern and eastern. *C. eremites* also had little variation but formed Holarctic and Nearctic clades. *C. pallipes* and *C. ornithophila* were very divergent from each other and from all other species. *C. pecuarum* was polyphyletic, some individuals falling within the *C. dacotensis* clade but was never found sympatric with *C. dacotensis*. *C. dacotensis* and *C. ornithophilia* were collected in complete sympatry in Kansas, Pennsylvania, and Ontario.

EXPANDED SURVEYS AND NEW SPECIES OF BLACK FLY GUT FUNGI IN SOUTHERN IDAHO

Nicole Reynolds[+], Emma Wilson, Prasanna Kandel, Matthew Laramie and Merlin White
Boise State University, Department of Biological Sciences, 1910 University Drive, Boise, Idaho, 83725
[+]Corresponding author email: nicolereynolds1@u.boisestate.edu

Trichomycetes (gut fungi) are obligate symbionts of various arthropods and have been found in marine, freshwater and terrestrial habitats on every continent except Antarctica. Minimally, gut fungi associate commensally with their immature aquatic hosts (including black flies, mayflies, stoneflies, isopods, and others) attaching to the chitinous lining of the mid- or hindgut, although relationships may shift depending on the situation. Both the geographic distribution and the biodiversity of gut fungi are vastly underestimated. Southern Idaho is no exception, as it presents many opportunities for discovery in unique habitats, including the sagebrush steppe. Fourteen locations in remote forest streams of Idaho were surveyed for macroinvertebrates, which were immediately preserved in 95% ethanol. Upon inspection, specimens occasionally had gut fungi protruding from the anus. Rehydration and microdissection techniques were utilized to dissect, slide mount and identify the gut fungi. Of the black fly larvae sampled, all were infested with *Harpella* in the midgut. Interestingly, a new species of *Genistellospora*, which has unusually large tricho- and zygospores, and is being described from a separate, less remote sampling location, was observed in many of the samples. From the data collected at these sites, it is clear that habitats in Idaho provide a healthy diversity of trichomycetes. Future studies will continue to increase our knowledge of this widespread group of endosymbionts.

A SIDE ORDER OF FLIES: UPDATE ON THE BITING FLY COMPONENT OF THE NORTHERN BIODIVERSITY PROGRAM

Patrick Schaefer[1,2] and Douglas C. Currie[1,2]
[1]Ecology and Evolutionary Biology, University of Toronto, Toronto, ON, Canada
[2]Department of Natural History, Royal Ontario Museum, Toronto, ON, Canada

Arctic and Boreal regions are among the most fragile ecosystems on Earth. Recently, they have come under immense environmental pressure as the effects of global warming are felt most acutely at northern latitudes. The Northern Biodiversity Program (NBP) is an integrative and strategic approach to understanding how biodiversity has and is adapting to changing environments. In 2010 and 2011, teams of entomologists from three universities revisited 12 sites across northern Canada that were originally sampled during the 1947-1962 Northern Insect Survey (NIS). As part of this program, our research focuses on arguably the most renowned residents of the north - the biting flies. Preliminary faunistic trends are presented as well as some tantalizing evidence which suggests that at least some biting flies may be shifting their distribution northwards in response to a changing northern climate. The utility of DNA barcoding for exploring species boundaries and phylogeographic patterns is discussed.

ANOTHER REMNANT SITE FOR THE ANCESTOR OF *S. SAXOSUM* AND *S. ARCTICUM* S.S.

Gerald F. Shields, Department of Biological Sciences, Carroll College, Helena, MT, 59601

Previous cytogenetic research indicates the presence of a remnant ancestor to *S. saxosum* and *S. arcticum* s.s. at the Coeur d'Alene River in Northern Idaho (Shields and Kratochvil, 2011, Amer. Mid. Nat.). No other remnant sites were discovered in an east to west collection scheme. In 2011 I sampled nearby drainages to the north and south of the Coeur d'Alene and discovered remnant sites at Wolf Lodge Creek and at the St. Joe River 50 km. to the south. As with the Coeur d'Alene site, the St. Joe site has larvae typical of *S. saxosum* (IIL-2 males and females), of *S. arcticum* s. s. (IIL-3 males and females) and combinations of the two. This indicates that populations ancestral to *saxosum* to the west and *arcticum* s. s. to east still remain. Moreover, a cytotype new to science (*S. arcticum* IIL-79) occurs in abundance at the St. Joe and, like larvae at the Coeur d'Alene, IIL-79 occurs in combinational forms with *saxosum* and *arcticum* s. s. These studies indicate that chromosomal inversions, particularly those linked to the y-chromosome in larvae of the *S. arcticum* complex play an early and important role in population differentiation.

DEFINING THE CYTOTYPE AND PERSISTENCE CONCEPTS IN BLACK FLY CYTOGENETICS

Jeanna Van Hoey and **Gerald F. Shields**, Department of Biological Sciences, Carroll College, Helena, MT, 59601

Chromosomal inversions play an important role in the differentiation of populations of black flies. That is, most "morphospecies" of black flies are complexes of sibling species differentiable only through cytogenetic analysis. At what point do we define types as siblings (good species) or merely cytotypes (types differentiable only by unique, y-linked chromosomal inversions)? And do these types persist at the same site year-after-year? We analyzed nearly 1,000 larvae from a single collection from the Little Blackfoot River, Montana and compared cytogenetic results to smaller collections previously taken from the same site. With some exceptions, we found that types previously described continue to exist at the Little Blackfoot but that new, previously unknown cytotypes occur when larger sample

sizes are analyzed. This suggests that many more cytotypes may exist at sites than may be indicated by casual analysis of fewer individuals.

TWELVE YEARS OF CYTOGENETIC ANALYSIS OF THE *S. ARCTICUM* COMPLEX IN WESTERN MONTANA; CORRELATIONS WITH ENVIRONMENTAL AND BIOLOGICAL FACTORS

Gerald F. Shields, Department of Biological Sciences, Carroll College, Helena, MT, 59601

For the past 12 years my research students and I have analyzed the cytogenetics of nearly 15,000 larvae of the *Simulium arcticum* complex from 56 geographic sites and 244 collections. In this presentation, we compare diversity of types, linkage to y-chromosomes, distributions, co-emergence of types, emergence times, drainage patterns, water temperatures, overall cytogenetic diversity and frequencies of very rare types across the complex. We seek additional suggestions on types of analyses.

DEVELOPMENT OF AN AREA-SPECIFIC PICTORIAL KEY FOR STREAMLINED LARVAL BLACK FLY MONITORING EFFORTS

Kristin E. Sloyer and John R. Wallace, Millersville University, Millersville, PA

Because of the homogeneity of morphology within the family, Simuliidae, black fly larvae are notoriously difficult to identify based on morphological characteristics alone. However, careful inspection of key morphological characteristics and knowledge of habitat can help to distinguish between certain species with success. The Hunterdon County Department of Vector Control desired to monitor the species of the South Branch of the Raritan River for scientific purposes, in particular, to assess the distribution of *Simulium jenningsi* compared to non-pest species throughout the river. However use of large all-encompassing keys proved to be impractical for the small number of species known to occur in the South Branch of the Raritan River, and local keys did not typically include all of the prevalent species. In order to identify species more efficiently, a regional black fly species identification key was formulated. We chose to focus primarily on characteristics such as head pigmentation, rectal gill characteristics, and hypostomal cleft shape in order to cut back on the use of high magnification microscopy, thereby streamlining the identification process. It is anticipated that the upcoming black fly monitoring season will be greatly improved by the development of this key, allowing the Hunterdon County Vector control Program to better serve the county's black fly pest problem.

METROPOLITAN MOSQUITO CONTROL DISTRICT (MMCD) BLACK FLY PROGRAM UPDATE WITH NATIONAL POLLUTANT DISCHARGE ELIMINATION SYSTEM (NPDES) PERMIT UPDATE

John Walz, Metropolitan Mosquito Control District, St. Paul, MN

The goal of the Metropolitan Mosquito Control District (MMCD)'s Black Fly Program is to reduce pest populations of black flies within the MMCD to tolerable levels. The MMCD monitors over 150 small stream sites and 28 large river sites in the 7-county metropolitan area surrounding Minneapolis-St. Paul each year. An update of the 2011 season will be discussed plus the implications of the National Pollutant Discharge Elimination System (NPDES) permit application process for the 2012 treatment season.

NON-TARGET EFFECTS OF FUNGICIDES ON BLACK FLY ASSOCIATED GUT FUNGI AND ACCUMULATION OF PESTICIDES IN LARVAL TISSUE

Emma R. Wilson[1*], Kelly L. Smalling[2], Timothy J. Reilly[3], Lance Steele[1], Prasanna Kandel[1], Alison B. Chamberlin[1], Justin W. Gause[1], and Merlin M. White[1]

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Fungicides of various forms have long been used to control fungal diseases and play an important role in modern agriculture. However, they tend to be understudied and are not typically included in water quality monitoring programs. Fungicides are moderately hydrophobic and their persistence has been documented in water and sediment as a result of non-point source pollution. It has recently been shown that the test organisms used to monitor fungicides are not sensitive enough to protect non-target fungal communities. To determine effects of fungicides on non-target trichomycetes (gut fungi), from two agricultural and two reference surface water sites were sampled for black fly larvae between April and December 2010. Surface water samples were collected to determine pesticide concentration. Black fly associated gut fungi were assessed, and larval tissue was tested for fungicide accumulation. Trichomycetes were observed to have lower prevalence, density, and fecundity in agriculture sites compared to reference sites. Preliminary results indicate that fungicides may have the potential to affect non-target fungal communities in surface water systems. Ongoing research is focusing on microcosms to determine at what concentration of specific fungicides trichomycetes and their hosts are impaired.