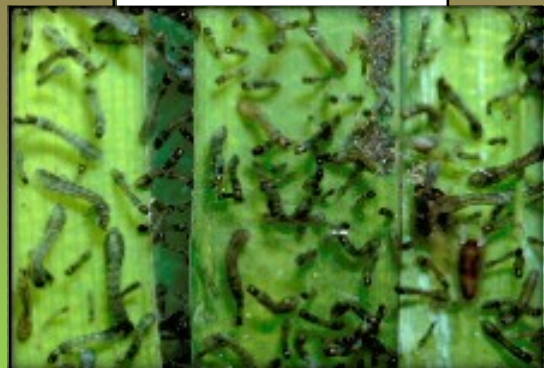


NORTH AMERICAN BLACK FLY ASSOCIATION



11TH ANNUAL MEETING

FEBRUARY 9TH – 10TH, 2013



PROGRAM AGENDA

UNIVERSITY OF GEORGIA • ATHENS, GEORGIA

SCHEDULE OF PRESENTATIONS AND EVENTS

SATURDAY, FEBRUARY 9TH

- 8:30 AM – 8:40 AM WELCOME TO THE GEORGIA CENTER: INTRODUCTIONS AND UPDATES
John Walz, Metropolitan Mosquito Control District, St. Paul, MN
- 8:40 AM – 9:10 AM BURULI ULCER DISEASE: AN EMERGING INFECTIOUS DISEASE WHERE TRANSMISSION REMAINS A MYSTERY
Richard W. Merritt, University Distinguished Professor, Department of Entomology, Michigan State University, East Lansing, MI
- 9:10 AM – 9:30 AM THE NORTH AMERICAN LARVAL BLACK FLY DATABASE
John W. McCreadie¹ and Peter Adler²
¹University of South Alabama, Mobile, AL
²Clemson University, Clemson, SC
- 9:30 AM – 9:50 AM THE CONTINUING MYSTERY OF TRICHOMYCETE HOST SELECTION: BLACK FLIES VS. MOSQUITOES
Charles E. Beard, Clemson University, Clemson, SC
- 10:00 AM – 10:30 AM **BREAK**
- 10:30 AM – 11:10 AM THE SIGNIFICANCE OF CHROMOSOME CHANGE IN THE SPECIATION PROCESS IN THE *SIMULIUM ARCTICUM* COMPLEX OF BLACK FLIES (DIPTERA: SIMULIIDAE)
Gerald F. Shields, Department of Biological Sciences, Carroll College, Helena, MT
- 11:10 AM – 11:30 AM *AUSTROSIMULIUM LONGICORNE*, NEW ZEALAND'S SLOW FLOW BLACK FLY
Douglas A. Craig, Professor Emeritus Exercens, Department of Biological Sciences, University of Alberta, Edmonton, Alberta, CANADA
- 11:30 AM – 11:50 AM LARVAL BLACK FLY MORTALITY FOLLOWING *BtI* EXPOSURE, IMPLICATIONS FOR POST-TREATMENT EVALUATIONS
Joseph Iburg, University of Georgia, Athens, GA
- 12:00 PM – 1:30 PM **LUNCH (PROVIDED AT GEORGIA CENTER)**
- 1:30 PM – 1:50 PM GAMMA AND TAXONOMIC DIVERSITY OF PREIMAGINAL BLACK FLIES (DIPTERA: SIMULIIDAE) OF THE MID AND EASTERN UNITED STATES
Rachel Williams¹, John W. McCreadie¹, Peter Adler², and Sam Stutsman¹
¹University of South Alabama, Mobile, AL
²Clemson University, Clemson, SC

SATURDAY, FEBRUARY 9TH (CONTINUED)

- 1:50 PM – 2:10 PM **SECRETED PROTEINS PRODUCED BY *SIMULIUM DECORUM* SILK GLANDS**
Patrick Viel, Brock University, St. Catherines, Ontario, CANADA
- 2:10 PM – 2:30 PM **CLIMATE-DRIVEN CHANGES IN ARCTIC BLACK FLY COMMUNITIES**
Patrick Schaefer^{1,2} and Douglas C. Currie^{1,2}
¹Ecology and Evolutionary Biology, University of Toronto, Toronto, ON, CANADA
²Department of Natural History, Royal Ontario Museum, Toronto, ON, CANADA
- 2:30 PM – 2:50 PM **OVIPOSITION AND HOST-SEEKING BEHAVIORAL RESPONSES OF *SIMULIUM VITTATUM* IS-7 (DIPTERA: SIMULIIDAE) TO POTENTIAL ATTRACTANTS IN LABORATORY BIOASSAYS**
Tommy W. McGaha Jr.¹, Ray Noblet¹, Thomas Unnasch², and Sayed Hassan³
¹Department of Entomology, College of Agricultural and Environmental Sciences, University of Georgia, Athens, Georgia
²Department of Global Health, University of South Florida, Tampa, Florida
³Department of Crop and Soil Sciences, College of Agricultural and Environmental Sciences, University of Georgia, Athens, GA
- 3:00 PM – 3:30 PM **BREAK**
- 3:30 PM – 3:50 PM **RAPID DIVERSIFICATION OF MOSQUITO AND BLACK FLY SALIVARY PROTEINS IS LIKELY DRIVEN BY HOST IMMUNE RESPONSES**
Donald E. Champagne, Department of Entomology, University of Georgia, Athens, GA
- 3:50 PM – 4:10 PM **POPULATIONS OF BLACK FLIES ON THE LANDSCAPE OF AN ENDANGERED SPECIES**
Peter H. Adler¹ and Elmer W. Gray²
¹Clemson University, Clemson, SC
²Department of Entomology, University of Georgia, Athens, GA
- 4:10 PM – 4:40 PM **TWO YEARS OF BLACK FLY SUPPRESSION IN SUPPORT OF THE ENDANGERED WHOOPING CRANE**
Elmer W. Gray¹, Peter H. Adler², and John Smink²
¹Department of Entomology, University of Georgia, Athens, GA
²Clemson University, Clemson, SC
- 4:40 PM – 5:00 PM **PENNSYLVANIA BLACK FLY SUPPRESSION PROGRAM UPDATE**
David Rebutck, Pennsylvania Department of Environmental Protection, Division of Vector Management, Black Fly Suppression Program
- 5:00 PM **PRESENTATION OF *MIKE SPIRONELLO AWARD***
FOR OUTSTANDING STUDENT PRESENTATION
- 5:30 PM **GROUP PICTURE**
- 6:00 PM **BBQ DINNER AND TOUR OF RIVERBEND RESEARCH LABS-HOME OF THE BLACK FLY COLONY**
(TRANSPORTATION WILL BE PROVIDED)

SUNDAY, FEBRUARY 10TH

AFTERNOON

OPTIONAL ACTIVITIES (DISTANCE FROM GEORGIA CENTER):
STATE BOTANICAL GARDEN (10 MIN. VAN RIDE)
GEORGIA ART MUSEUM (1/3 MILE WALK)
GEORGIA LADY DAWGS SOFTBALL GAME (10 MIN VAN RIDE,
GAME IS AT 12:30PM)

Thanks to the Georgia Center for hosting our NABFA meeting!

*President: John Walz, Vice-President: Elmer Gray
Program editor: Carey LaMere, T-shirt design: Marty Kirkman*

NABFA WEBSITE: www.nabfa-blackfly.org





11TH ANNUAL NABFA MEETING

PRESENTATIONS TITLES WITH ABSTRACTS

FEBRUARY 9 – 10, 2013

UNIVERSITY OF GEORGIA, ATHENS, GEORGIA

(In alphabetical order by presenter)

POPULATIONS OF BLACK FLIES ON THE LANDSCAPE OF AN ENDANGERED SPECIES

Peter H. Adler¹ and Elmer W. Gray²

¹Clemson University, Clemson, SC

²Department of Entomology, University of Georgia, Athens, GA

Population dynamics of black flies in the nesting habitat of endangered cranes are summarized and interpreted for a five-year period, including before and after a suppression program using *Bti* to target the principal ornithophilic pest species of the cranes.

THE CONTINUING MYSTERY OF TRICHOMYCETE HOST SELECTION: BLACK FLIES VS. MOSQUITOES

Charles E. Beard, Clemson University, Clemson, SC

We are continuing to explore the colonization potential of mosquito midguts by a trichomycete fungus (*Harpella melusinae*). This fungus is not culturable, so we developed a system to collect live spores in water from field-collected black flies. The spores are then used to attempt colonization of mosquito midguts. We previously reported that field-collected *Harpella melusinae* can germinate, attach, and grow in *Aedes albopictus* larvae, but no fungal spores were produced. Lab-reared *Culex* species also supported limited germination and attachment of *Harpella melusinae*. Lab-reared *Aedes aegypti* has so far not supported detectable attachment of *Harpella melusinae*. There remain multiple as yet unanswered questions. Principally, why has no trichomycete fungus species developed to use the mosquito midgut as a habitat? Trichomycetes commonly inhabit other aquatic Diptera midguts. *Harpella melusinae* will germinate and grow in some mosquito midguts in the lab; it is common in, but limited to, black flies in nature. What is the mechanism or physiology that restricts it to the family Simuliidae in nature? Is *Aedes albopictus* more susceptible to symbiotes than other mosquito species? Are there undiscovered midgut trichomycetes in *Aedes albopictus* in its native areas? Will the trichomycetes *Stachylina* spp., which commonly grow in midguts of lentic and lotic chironomids, colonize mosquito midguts?

RAPID DIVERSIFICATION OF MOSQUITO AND BLACK FLY SALIVARY PROTEINS IS LIKELY DRIVEN BY HOST IMMUNE RESPONSES

Donald E. Champagne, Department of Entomology, University of Georgia, Athens, GA

No abstract

AUSTROSIMULIUM LONGICORNE, NEW ZEALAND'S SLOW FLOW BLACK FLY

Douglas A. Craig, Professor Emeritus Exercens, Department of Biological Sciences, University of Alberta, Edmonton, Alberta, CANADA

The New Zealand black fly *Austrosimulium longicorne*, of which the larvae have unusual habitat requirements is highlighted. Larvae prefer very smooth, low-velocity flow, down to 10 cm/ sec and as shallow as three millimeters. Iron bacteria-laden seeps can harbour vast numbers of larvae and in such places appear to be the only macroinvertebrate present. Such habitats can be detected using Google Earth, which presents a novel manner for finding localities.

TWO YEARS OF BLACK FLY SUPPRESSION IN SUPPORT OF THE ENDANGERED WHOOPING CRANE

Elmer W. Gray¹, Peter H. Adler², and John Smink²

¹Department of Entomology, University of Georgia, Athens, GA

²Clemson University, Clemson, SC

No abstract

UNIVERSITY OF GEORGIA RESEARCH DEMONSTRATES STREAM WATER QUALITY MAINTAINED AS BROILER LITTER USED FOR PASTURE FERTILIZATION

Nancy Hinkle, Department of Entomology, University of Georgia, Athens, GA

No abstract

LARVAL BLACK FLY MORTALITY FOLLOWING *Bti* EXPOSURE, IMPLICATIONS FOR POST-TREATMENT EVALUATIONS

Joseph Iburg, University of Georgia, Athens, GA

The effectiveness of *Bti* applications for the control of black fly larvae is often determined with a post-treatment evaluation. Larvae are removed from the substrate and the mortality is calculated. The time necessary for the effects of *Bti* to manifest in all exposed larvae can vary, and there is no consensus on how long to wait before evaluating the effects. *Simulium vittatum* larvae were exposed to *Bti* and larval mortality was monitored over time. Larvae exposed to operational concentrations of *Bti* exhibited maximum mortality, approximately 90%, 2 hours post-treatment. Larvae exposed to 1/3 of that concentration exhibited the same mortality after 7 hours. Additional experiments revealed that maximum mortality and time required to achieve maximum mortality can be affected by components in the larval medium. Larval mortality was compared between larvae exposed to *Bti* in clean water, 50 ppm of clay (40 NTU), or 50 ppm of cellulose (6.5 NTU). All larvae received the same operational concentration of *Bti*. The clay had no observable effects on larval mortality or time until maximum mortality. Cellulose in the larval medium resulted in a greater than 50% increase in time to maximum mortality and greater than 40% reduction in overall mortality.

LONG-TERM NON-TARGET MONITORING FOR LARVAL BLACK FLY CONTROL OPERATIONS IN THE MISSISSIPPI RIVER

Carey LaMere and John Walz, Metropolitan Mosquito Control District, St. Paul, MN

The Metropolitan Mosquito Control District (MMCD) in Minnesota conducts biennial monitoring of the non-target invertebrate population in the Mississippi River as part of the larval black fly control permit requirements set by the Minnesota Department of Resources (MnDNR). This monitoring began in 1995. The study was designed to provide a long-term assessment of the invertebrate community in *Bacillus thuringiensis israelensis* (*Bti*)-treated reaches of the Mississippi River. Results from monitoring data collected and analyzed through 2009 indicate that there have been no large-scale changes in macroinvertebrate community in the *Bti*-treated reaches of the Mississippi River.

THE NORTH AMERICAN LARVAL BLACK FLY DATABASE

John W. McCreddie¹ and Peter Adler²

¹University of South Alabama, Mobile, AL

²Clemson University, Clemson, SC

The purpose of this project is to compile all published and unpublished larval black fly collection records from North America (north of Mexico) into a single, geo-referenced database. In addition, all parasite data for each collection is also entered. To date over 3000 collections have been entered into the database, including all sites in the US east of the Mississippi. This data base provides a means to ask broad questions in black fly ecology, biogeography, systematics and control. Examples of the database and its applications are given.

3 GENOMES PLUS A TRANSCRIPTOME: GENOMIC DATA AVAILABLE FOR THE BLACK FLY COMMUNITY / BLACK FLIES OF THE GALAPAGOS ISLANDS

Charles Brockhouse¹ and **John W McCreadie**²

¹Creighton University, Omaha, NE

²University of South Alabama, Mobile, AL

No abstract

OVIPOSITION AND HOST-SEEKING BEHAVIORAL RESPONSES OF *SIMULIUM VITTATUM* IS-7 (DIPTERA: SIMULIIDAE) TO POTENTIAL ATTRACTANTS IN LABORATORY BIOASSAYS

Tommy W. McGaha Jr.¹, Ray Noblet¹, Thomas Unnasch², and Sayed Hassan³

¹Department of Entomology, College of Agricultural and Environmental Sciences, University of Georgia, Athens, Georgia

²Department of Global Health, University of South Florida, Tampa, Florida

³Department of Crop and Soil Sciences, College of Agricultural and Environmental Sciences, University of Georgia, Athens, GA

The oviposition and host-seeking responses of *Simulium vittatum* IS-7 (Diptera: Simuliidae) to potential attractants were investigated in laboratory bioassays. The response of gravid *S. vittatum* IS-7 in a binary choice chamber demonstrated that fresh conspecific eggs stimulated oviposition. Another set bioassays, oviposition attraction bioassays, focused more on observing the behavior of the gravid females to the volatiles of the eggs, which demonstrated no attraction. A petri dish bioassay demonstrated that gravid *S. vittatum* IS-7 were stimulated to oviposit by a water or hexane extract of conspecific eggs quicker than a control. Gas chromatography-mass spectrometry was used to identify potential oviposition stimulation factors in the active extracts. For observing host-seeking behavior, a new Y-tube olfactometer was developed to investigate parous *S. vittatum* IS-7 in efforts to identify host-seeking attractants. This series of bioassays have helped the understanding of black fly oviposition and host seeking behavior.

BURULI ULCER DISEASE: AN EMERGING INFECTIOUS DISEASE WHERE TRANSMISSION REMAINS A MYSTERY

Richard W. Merritt, University Distinguished Professor, Department of Entomology, Michigan State University, East Lansing, MI

No abstract

PENNSYLVANIA BLACK FLY SUPPRESSION PROGRAM UPDATE

David Rebuck, Pennsylvania Department of Environmental Protection, Division of Vector Management, Black Fly Suppression Program

No abstract

WEST VIRGINIA BLACK FLY CONTROL PROGRAM UPDATE

Betsy Reeder, WV Department of Agriculture, Charleston, WV

No abstract

CLIMATE-DRIVEN CHANGES IN ARCTIC BLACK FLY COMMUNITIES

Patrick Schaefer^{1,2} and Douglas C. Currie^{1,2}

¹Ecology and Evolutionary Biology, University of Toronto, Toronto, ON, CANADA

²Department of Natural History, Royal Ontario Museum, Toronto, ON, CANADA

Unprecedented warming trends at high latitudes are expected to promote the northward dispersal of deleterious species into habitats previously naive to these stresses. However, detecting distributional changes is often difficult due to lack of adequate baseline data. Fortunately, black flies were the focus of intense study during the half-century-old Northern Insect Survey (NIS). This extensive sampling revealed only 2 species of arctic black

flies are capable of haematophagy and both are restricted to southern Baffin Island. The Northern Biodiversity Programs (NBP) is an integrative and strategic approach to understanding how biodiversity has and is adapting to changing environments. The NBP resampled twelve of the original NIS sites in 2010-2012 to document what changes, if any, transpired over a half century time scale. Alarming, our collections nearly doubled the number of black fly species found in the Canadian Arctic Archipelago, of which almost half are bloodsucking pests of mammals and birds. The greatest increase in species richness was observed on southern Victoria Island. Extensive sampling of Victoria Island in 2012 showed a strong association between temperature regime and the distribution of blood-feeding species. Using the contemporary thermal limits of biting species, we are able to more confidently determine when biting species first invaded Victoria Island, and predict how long it will take for the island to become completely colonized.

THE SIGNIFICANCE OF CHROMOSOME CHANGE IN THE SPECIATION PROCESS IN THE *SIMULIUM ARCTICUM* COMPLEX OF BLACK FLIES (DIPTERA: SIMULIIDAE)

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

This study summarizes cytogenetic variation, particularly sex-linked chromosomal inversions which define taxa of the *Simulium arcticum* complex (Diptera: Simuliidae) in western Montana and portions of northern Idaho, Washington state and Oregon. Variation in polytene chromosomes was determined for nearly 15,000 larvae from 234 collections taken from 60 fresh-water sites. The previously described siblings: *S. apricarium*, *S. arcticum* sensu stricto, *S. brevicercum*, and *S. saxosum*, were most numerous, while all cytotypes with the exception of IIL-19 were found in low frequency. Additionally, six new cytotypes in low frequency are described. Evidence suggests that the Y chromosome carries the testis-determining gene and in almost all taxa of *S. arcticum* complex paracentric inversions characterize these types. Distributions of: 1) *S. brevicercum* and *S. arcticum* s. s., of 2) *S. arcticum* s. s. and *S. arcticum* IIL-18, of 3) *S. brevicercum* and *S. arcticum* IIL-18, and of 4) *S. arcticum* IIL-9 and *S. arcticum* IIL 19 have highly significant positive geographic associations while those of: 1) *S. apricarium* and *S. brevicercum* and of 2) *S. apricarium* and *S. saxosum* have highly significant negative geographic associations. The *S. arcticum* cytotypes: IIS-12, IIL- 17, IIL-21, IIL-22, IIL-38, IIL-51, IIL-68, IIL-73•74 and IIL-79 occur only at two or fewer locations. Polyploids (0.0007), pericentric inversions (0.00007) and chromosomal translocations (0.00007) are exceedingly rare. These observations and our recent DNA comparisons of chromosomally distinct types lead me to elaborate on a previously suggested model for chromosome evolution in black flies in which locally distributed cytotypes may become more common with time while wide-spread cytospecies may eventually become morphologically differentiated types. Contrary to the current understanding that chromosome variation may not play a significant role in the speciation process of most animals, this study suggests that chromosomal variation, at least in black flies, plays a significant role in speciation.

THE CYTOGENETICS OF *SIMULIUM DEFOLIARTI*

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

This study summarizes biological and cytogenetic variation (including sex-linked variation) in two populations of *Simulium defoliarti* at 1) Icicle Creek, Chelan Co. in central Washington state (47° 33' 49" N, 120° 40' 20" W) and 2) the Boulder River, Jefferson Co. 112° 07' 20" N, 46° 24' 10" W in western Montana. Sexual dimorphism in larval color, fixation of the IIS14•15 inversion, centromere dimorphism (enhanced vs. thin), and paracentric inversion linkage to the Y chromosome were determined. This study serves as an introduction to further cytogenetic and biological studies of this little known but close relative to the *S. arcticum* complex.

TWIN FALLS COUNTY PEST ABATEMENT DISTRICT UPDATE

Kirk Tubbs, Twin Falls County Pest Abatement District, Twin Falls, ID

No abstract

SECRETED PROTEINS PRODUCED BY *SIMULIUM DECORUM* SILK GLANDS

Patrick Viel, Brock University, St. Catharines, Ontario, CANADA

Black fly (Simuliidae) larvae use silk as safety lines or as pads to anchor themselves to the substrate. In addition, simuliid pharate pupae spin cocoons for protection. There is very little information about the composition of simuliid silk proteins. In this study we present preliminary data suggesting that the larval and pharate pupal salivary glands produce a wide array of proteins. Salivary glands of larvae and pharate pupae were dissected out and incubated in extraction buffer. Crude protein extracts were separated using SDS-PAGE and gels were stained with either silver or periodic acid Schiff stains. There are over 10 different proteins produced by salivary glands, ranging in size from <40 kDa to >300 kDa. The protein profile is similar between larvae and pharate pupae. PAS stain confirms glycosylation only in the largest protein. We have identified a large array of proteins. Future studies will focus on interspecific comparisons of silk proteins and also identifying the protein and gene sequences. These sequences will give insight into the specific functions of the proteins, and eventually help to establish a better understanding of the evolution of these genes throughout the entire family. In addition, this genetic information can help to contribute to downstream applications involving the foreign expression and mass production of these silk proteins.

METROPOLITAN MOSQUITO CONTROL DISTRICT (MMCD) BLACK FLY CONTROL PROGRAM UPDATE

John Walz and Carey LaMere, Metropolitan Mosquito Control District, St. Paul, MN

The goal of the Metropolitan Mosquito Control District (MMCD)'s Black Fly Control Program is to reduce pest populations of black flies within the MMCD to tolerable levels. The MMCD monitors 165 small stream sites and 28 large river sites in the 7-county metropolitan area surrounding Minneapolis-St. Paul each year. An update of the 2012 season will be discussed.

A STATISTICAL ANALYSIS OF CULICOMORPHA-*SMITTIUM* COEVOLUTION

Yan Wang¹, Merlin M. White², and Jean-Marc Moncalvo^{1,3}

¹Department of Ecology and Evolutionary Biology, University of Toronto, Toronto, ON, Canada

²Department of Biological Sciences, Boise State University, Boise, ID, USA

³Department of Natural History, Royal Ontario Museum, Toronto, ON, Canada

Found and described by Poisson in 1936, *Smittium* is one of the oldest members of the Harpellales, a group of endosymbiotic microorganisms that commonly live in the digestive tracts of various lower Diptera, thus referred to as the gut fungi. With 81 species, *Smittium* has served as the “model” and helped advance our understanding of the gut fungi, from aspects of biochemistry, biodiversity, ecology, evolution, immunology, and physiology. The hosts of this gut fungus genus include Chironomidae, Culicidae, Dixidae, Scirtidae, Simuliidae, Thaumaleidae, and Tipulidae. Recently a multigene phylogeny of *Smittium* (sensus lato), based on 18S and 28S rDNA, RPB1, RPB2, and MCM7 protein sequences, has been constructed (unpublished), revealing a major separation within the genus, which is also supported by certain morphological evidence (thallus branching type, holdfast shape, trichospore or zygosporangium characters). A phylogeny for the Culicomorpha has also been published (Wiegmann et al. PNAS 2011). However the host-endosymbiont link and the co-phylogenetic studies of *Smittium* species and their insect hosts have never been conducted. We present co-phylogenetic analyses between the Culicomorpha hosts and their *Smittium* endosymbionts, using UNIFRAC and the “parafit” function in the software R. Preliminary results will be presented.

GAMMA AND TAXONOMIC DIVERSITY OF PREIMAGINAL BLACK FLIES (DIPTERA: SIMULIIDAE) OF THE MID AND EASTERN UNITED STATES

Rachel Williams¹, John W. McCreadie¹, Peter Adler², and Sam Stutsman¹

¹University of South Alabama, Mobile, AL

²Clemson University, Clemson, SC

Total species diversity in a landscape (gamma diversity) is determined by two different things, the mean species diversity in sites or habitats at a local scale (alpha diversity) and the differentiation among those habitats (beta diversity) Whittaker (1960). The family Simuliidae is currently one of the most well-known taxonomic groups of aquatic insects at the species level. The goals of this study are to determine how species richness and distribution of larval black flies varies over large geographic landscapes and ecoregions and whether species richness and distribution can be predicted by stream variables and landscape features. Data obtained from field specimens will be used to create a GIS model of species richness and distribution of blackfly species. GIS (Geographic Information Systems) technologies are enabling scientists to analyze spatially extensive communities, gain insights on genetic diversity and population dynamics, and also for modeling geographical distances of organisms. Accordingly, data from this study will also aide in the identification of morphological features that could separate adult sibling (cryptic) species. Over 130 sites from Texas, Louisiana, Mississippi, Alabama, Florida and Arkansas have been sampled. The methodology for field collections is in accordance with McCreadie and Adler (1998). All samples have been sent to Dr. Peter Adler at Clemson University for genetic analysis.