The 5th Annual Meeting of the North American Black Fly Association (NABFA)

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The 5th Annual Meeting of the North American Black Fly Association was held 7-9 February 2007 at the University of Georgia, in Athens, Georgia. The meeting was chaired and organized by NABFA President Jay P. Overmyer. Thirty-four workers attended and 24 presentations were given. Topics included black fly control, ecology, taxonomy, systematics and biogeography.

Among the highlights of the meeting was a guided tour of the University of Georgia's Black Fly Colony, which has been maintained continuously for more than 20 years. Another notable event was the inaugural *Mike Spironello Award*, presented annually to the student giving the best talk. The award was established to honor the memory of former NABFA Secretary and black fly researcher, Mike Spironello, who passed away unexpectedly in 2006. This year's winner was Paul Smith of the University of Georgia, who delivered a co-authored presentation on the black fly vectored disease vesicular stomatitus (full title and authorship given below). Paul was presented with a copy of Adler, Currie, and Wood's monumental book *The Black Flies (Simuliidae) of North America*.

The location for the 6th NABFA Meeting (2008) has yet to be determined. Please visit the NABFA website (**http://www.zoo.utoronto.ca/nabfa/NABFA.html**) for updated information in the near future. NABFA invites all black fly researchers to join NABFA and to present their research at upcoming meetings. Students are especially encouraged to participate in the *Mike Spironello Award* competition. Membership is free of charge. Please visit the NABFA website or contact secretary Julio Rivera (**julior@rom.on.ca)** for further information.

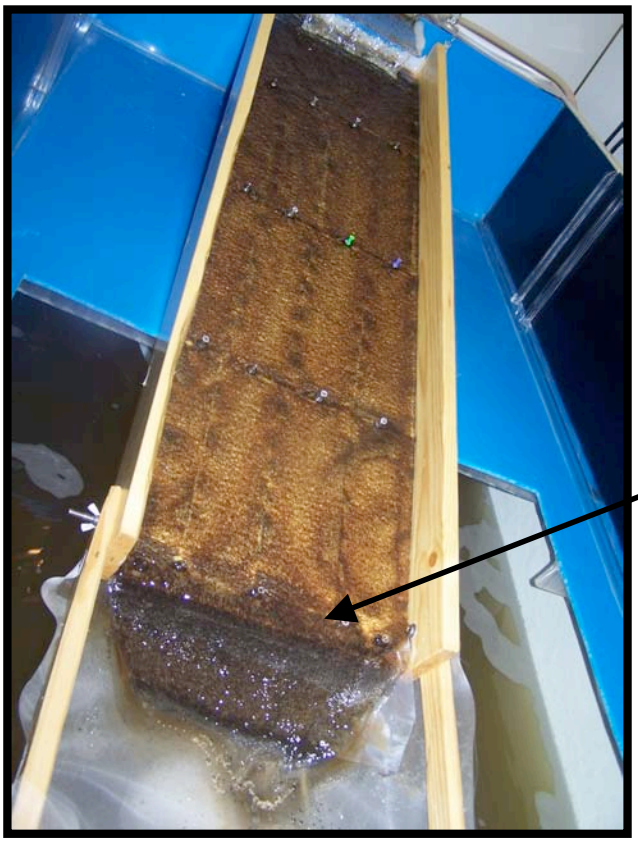
Presentations at the Georgia Continuing Education Center, University of Georgia

Dinner at UGA Bee Lab

Paul Smith, co-winner of the *Mike Spironello Award* receives a copy of the book "Black Flies of North America" from NABFA President, Jay Overmyer

Visit to the black fly colony (*Simulium vitattum*) kept at the University of Georgia

Elmer Gray explaining the rearing methodology



Thousands of larvae!

Regular Presentations:

REPRODUCTIVE STATUS OF CYTOSPECIES AND CYTOTYPES IN SYMPATRY AT THREE LOCATIONS IN WESTERN MONTANA**. Gerald F. Shields**, Judith A. Pickens, Lindee M. Strizich and Gregory M. Clausen Carroll College, Helena, MT.

Five sibling species (Shields and Procunier, 1982; Adler *et al*., 2004) and at least 10 cytotypes (Shields, unpub.) of the original morphospecies, *Simulium arcticum*, have been described. This substantial diversity begs the question of whether or not these taxa are reproductively isolated in sympatry. In our recent work in western Montana, cytotypes, populations having unique, sex-linked chromosomal inversions, generally have very limited geographic distributions and occur in sympatry with other cytospecies and cytotypes. Alternatively, cytospecies generally have broad continent-wide distributions. Assuming that cytospecies are old and cytotypes are young in an evolutionary sense, we investigated the reproductive status of taxa of the *S. arcticum* complex at three sites and hypothesized that cytospecies in sympatry would be reproductively isolated while cytotypes may not be. We used the distribution of autosomal polymorphisms to test for equilibrium at each of these sites and found that our hypothesis was generally supported.It seems clear that divergence in the *S. arcticum* complex is, in part, mediated by the origin of sex-linked, chromosomal inversions and further study of these taxa in sympatry may eventually lead to a model for radiation in this group.

THE BLACK FLY GENOME PROJECT PROPOSAL: PROGRESS REPORT. **Charles Brockhouse**. Creighton University, Omaha, NE (**Presented by Peter Adler**).

We propose a full genome sequencing project and an accompanying cDNA sequencing project for the Simuliidae (Black Flies). The full genome sequence will be an invaluable resource for the insect genomics community, allowing order-wide functional genomic comparative analysis of genomic contents and their organization, and functional analyses of critical parameters such as insect attributes linked to their capacity to transmit disease agents. These attributes include blood feeding (haematophagy), parasite/pathogen transmission, symbiosis, and insecticide resistance. The EST project is critical to assembling the full genome in the face of the absence of genetic maps and the presence of inversion polymorphisms, and will enormously enhance efforts to genetically map the black fly genome and explore gene regulation, differences among species, and symbiosis. The cDNA project is a critical component of this proposal, to facilitate the annotation of the genome sequence and to produce reliable microarrays that are used to explore the conservation of transcriptional regulators under conditions that are shared by species that vector the agents of disease. This project aims to make a significant impact in furthering genomic knowledge of vector biology, by promoting comparative research on a disease vector that has close phylogenetic relationships to both mosquitoes (*Anopheles*, *Aedes*) and non-haematophagous insects. Biological material will be supplied by participating laboratories (Noblet, Brockhouse, Adler, Post, McCreadie), while the CGB will carry out the genomic projects in support of the genome sequencing and efforts, including cDNA library construction/screening, sequence assembly validations, EST characterization, and

related bioinformatics. The principal investigators will solicit the involvement of a growing insect genomics research community for the overall analysis and annotation. The resulting database will be incorporated into VectorBase and within the proposed InsectBase.

WHAT'S GOING ON IN MINNESOTA? BLACK FLY MONITORING AND CONTROL IN THE GREATER METROPOLITAN AREA OF THE TWIN CITIES OF MINNEAPOLIS AND ST. PAUL, MINNESOTA, USA. **John Walz,** Abe Benson, Carey LaMere Metropolitan Mosquito Control District, Black Fly Control Program, St. Paul, MN.

In 1958, the Minnesota State Legislature created the Metropolitan Mosquito Control District (MMCD) to protect the public from disease and nuisance mosquitoes. In 1984, the MMCD was expanded to include a black fly control program for the seven-county metropolitan area surrounding Minneapolis and St. Paul. The Black Fly Control Program in Minnesota monitors and treats four major rivers and one hundred-fifty smaller stream locations that entwine the metropolitan area. Target species include *Simulium luggeri, S. meridionale, S. johannseni* and *S. venustum*. The Minnesota Department of Natural Resources (MDNR) is the legal authority in control of waterways in Minnesota. A permit, issued by the MDNR, is required to allow for treatment of black fly larvae. Non-target monitoring and adult monitoring are required to receive the permit.

PHYLOGENY OF THE BLACK FLY GENUS *Cnephia* (DIPTERA: SIMULIIDAE). **Kenneth Pruess,** University of Nebraska, Lincoln, NE.

Cnephia is a small Holarctic genus of black flies of probably 6 species. *C. pallipes* and *C. toptchievi* are Palearartic; *C. ornithophilia, C. dacotensis*, and *C. pecuarum* Nearctic; *C. eremites* Holarctic. Other named Russian species are likely synonyms of either *C. eremites* or *C. pallipes*. *C. pallipes* is considered the most primitive. Arrival in the Nearctic likely occurred in the Cretaceous. The ancestral species was most likely a bird feeder as is *C. ornithophilia*, the oldest extant Nearctic species. *C. eremites*, a non-feeding adult, retains the lobed claw of a bird feeder as does *C. pecuarum* which is a large mammal feeder. *C. dacotensis*, probably of most recent origin, has lost the lobed claw and is unusual among temperate species in being non-feeding as adults. A phylogeny is presented based largely on the mitochondrial CO-I gene for 5 species.

WHO'S IN CONTROL, THE FLY OR THE FUNGUS? SPORE SHAPE OF *Harpella melusinae* IN LARVAL BLACK FLIES. **Charles E. Beard** and Peter H. Adler**,** ESPS, Clemson University, Clemson, SC.

Black flies are the sole hosts of the trichomycete fungus *Harpella melusinae*, which is a common inhabitant of the larval midgut. *Harpella melusinae* can form asexual spores that are either straight or curved-coiled. Straight spores are always on thalli separate from

thalli with coiled spores, although thalli with different spore shapes can be found in the same host larva. Our objective is to determine if spore shape is a heritable character. We plan to test for horizontal transmission by exposing trichomycete-free larval black flies in the laboratory to trichomycetes of a given spore shape from field-collected larval black flies. This test will allow a comparison of the shapes of spores in newly colonized larvae. We expect that spore shape from field-collected larvae will be conserved in transmission to laboratory-reared flies. If so, the experiment would suggest that spore shape represents distinct genetic types, instead of host or environment-induced variation.

AN OVERVIEW OF BLACK FLY CONTROL IN HUNTERDON COUNTY, NEW JERSEY. **Tadhgh Rainey** Hunterdon County Department of Health, Flemington, NJ.

In 2001 a black fly larval control program was instituted in Hunterdon County, New Jersey to reduce *Simulium jenningsi* flies. The objective of this program was to implement thorough integrate pest management (IPM) strategies while maintaining sufficient levels of control. Bionomics of this species in northwest New Jersey are discussed. In addition, surveillance procedures and plans for improvement of IPM methods are addressed.

BLACK FLY VIGNETTES. **Peter H. Adler,** Clemson University, Clemson, SC.

The total number of described species of black flies recently surpassed 2,000, with the most species in the Palearctic Region (35%) and the fewest in the Afrotropical Region (11%); the Nearctic Region is home to about 13% of the world's species. Prospecting in various parts of the world, as well as within the black fly genome, continues to reveal new species and their relationships. Examples of recent discoveries are highlighted, including relationships of the Palearctic *Simulium variegatum* species group.

The contribution of South- and Central American lineages of black flies (Diptera: Simuliidae) to the North American fauna. **Douglas C. Currie**[1,2] and Justin Ancheta[2], [1]Royal Ontario Museum, Toronto, ON [2] University of Toronto, Toronto ON.

In contrast to the strong faunal similarity between the Nearctic and Palearctic Regions, which share a total of 18 genus-group taxa, only 6 such taxa are shared between the Neactic and Neotropical Regions. These latter lineages, which probably originated in South- and Central America, include representatives of the following genus-group taxa: *Tlalocomyia*, *Gigantodax*, *Psilopelmia*, *Psilozia*, *Aspathia*, and *Hemicnetha*. Collectively, these lineages contribute 39 of 256 species currently recognized from the Nearctic Region, representing about 15% of the total simuliid fauna of that region. Most of these species are variously distributed in western North America, whereas others are strictly eastern or widespread. Distributional patterns of these southern lineages are interpreted in view of geological- and climatic events from the late Miocene onwards.

STABLE ISOTOPE TURNOVER IN BLACK FLY, *Simulium vittatum* IS-7, LARVAE. **Jay Overmyer**[1], M. Aaron MacNeil[2] and Aaron T. Fisk[3], [1]University of Georgia, Athens,

GA, [2]University of Newcastle, Newcastle upon Tyne, UK, [3]University of Windsor, Windsor, ON.

Diet-tissue fractionation factors and metabolic turnover rates (*m*) of _^{15}N and _^{13}C were assessed in laboratory-reared black fly (*Simulium vittatum* IS-7) larvae fed isotopically-distinct diets. Five, 26 day experiments were carried out, three consisted of the same food (although the characteristics of the food varied) throughout the experiment but with different _^{15}N signatures, a fourth shifted from a low to high stable isotope signature diet (*uptake*) on day 14, and the last shifted from a high to low stable isotope signature diet (*elimination*) on day 14. The _^{15}N diet-tissue fractionation factors were generally negative, ranging from +2.85 to -24.96‰ and were highly influenced by the _^{15}N value in the food. The only positive value was from the *elimination* experiment and _^{15}N values in the larvae were not in steady-state with the food. The _^{15}N of shed head capsules collected during the low and high mixed food experiments were 1.2 and 7.1‰ higher than the larvae, respectively, and could be partially responsible for the negative fraction factors observed in the larvae. These factors became more negative with increasing _^{15}N values in the food suggesting that diet-tissue fraction factors are concentration-dependent, which would influence interpretation of _^{15}N in the environment. Results from Hesslein's model showed *m* for _^{15}N was consistent (0.40 to 0.43 _^{15}N*d^{-1}) between uptake and elimination phases of the experiment and were an order of magnitude greater than growth rates, in contrast vertebrate species where estimates of *m* are much lower than growth rates. Rapid metabolic turnover of N in black fly larvae makes them an excellent indicator of short-term changes in nitrogen inputs to aquatic systems.

EFFECTS OF *Bacillus thuringiensis israelensis* (*Bti*) BLACK FLY TREATMENTS ON NON-TARGET ORGANISMS IN THE DELAWARE RIVER IN PENNSYLVANIA AND NEW JERSEY. **Dave Rebuck,** Pennsylvania Department of Environmental Protection, Division of Vector Management, Black Fly Suppression Program.

A six-year bio-monitoring study on the Delaware River examined the long-term effects of *Bacillus thuringiensis israelensis* (*Bti*) black fly treatments on non-target fish and aquatic macroinvertebrate populations. From 1996 to 2001, the Delaware River received an average of 8.5 treatments per season, with an annual mean of 10,576 gallons of VectoBac® 12AS to control *Simulium jenningsi* group black flies. Macroinvertebrate sampling procedures followed EPA Rapid Bioassessment Protocols. Fish were sampled quantitatively in riffle and run habitats with a backpack electro-fishing unit. Macroinvertebrate and fish populations remained abundant and diverse during the study. Sensitive aquatic insect populations appeared to be unaffected by repeated *Bti* treatments for black fly control. Macroinvertebrate metrics changed equally at treatment and control sites. The species composition, abundance, condition, population density, and biomass of the fish community were not measurably impacted by *Bti* treatments. Benthic forage fish consumed fewer black flies at treatment stations, but successfully switched to an abundance of food resources. The Delaware River ecosystem remained stable and productive during the non-target organism bio-monitoring study.

THE VECTOBAC 12AS STORY: HOW INDUSTRY, ACADEMIA, NGO'S AND GOVERNMENTS COLLABORATED TO DEVELOP THE PREMIER BLACK FLY CONTROL LARVICIDE. **Ernest Dankwa,** Valent Biosciences.
THE ROLE OF CHROMOSOME CHANGE IN THE EVOLUTION OF BLACK FLIES. **Gerald Shields,** Carroll College, Helena, MT.

This talk will hopefully bring a renewed focus to the role chromosome change plays in the divergence of black flies. Classical cytogenetic theory argues that chromosome variants should occur randomly and be transient in extant populations because of their "deleterious effects" particularly at meiosis. On the contrary, it is abundantly clear that chromosome changes, particularly paracentric inversions, are essentially ubiquitous in populations of Simuliids. This presentation will recall classical models, summarize current data for well- studied groups, especially the *Simulium arcticum* complex, and hopefully stimulate interest in suggesting new research approaches to better understand these phenomena in black flies.

MOLECULAR TOOLS AND THE STUDY OF BLACK FLY-BORNE PARASITES. **Will Reeves,** Gainesville State College, Oconee Campus.

Molecular techniques have been used as diagnostic and experimental tools in the study of mosquito-borne pathogens. While some of these techniques have been applied to black flies, the use of molecular tools in the study of black fly-borne pathogens has not been as widespread at they have been in other arthropod vectors. Molecular techniques were applied to field collected black flies to determine if they were exposed to protozoan parasites. I will discuss the results of a survey of wild caught black flies for protozoan parasites.

TWENTY-FIVE YEARS OF BLACK FLY COLONIZATION, 1981-2006. **Elmer Gray,** University of Georgia, Athens, GA.

Abstract not available

Poster Presentations:

A LONGITUDINAL ANALYSIS OF THE DISTRIBUTIONS OF AND AN ASSESSMENT OF THE REPRODUCTIVE STATUS OF TWO SIBLINGS OF THE *S. arcticum* Complex AT LITTLE PRICKLY PEAR CREEK, LEWIS AND CLARK COUNTY, MONTANA. **Gregory M. Clausen** and Gerald F. Shields Carroll College, Helena, MT.

Little Prickly Pear Creek was the single exception among five drainages upon which the *S. arcticum s. s.* high/ *S. apricarium* low hypothesis was based. The site was unique in

that the ratio of the two taxa was reversed from the previous observations at the other four drainages, i.e. *S. arcticum s. s.* predominated at the original low elevation site. We therefore studied this drainage at four equally spaced locations to determine if some other abiotic factor, such as water temperature, might influence the distribution of these cytospecies. Since colder water is usually found at higher elevations and its temperature increases as it moves to lower elevations, I hypothesized that as I sampled down stream the frequency of *S. apricarium* would increase. Shields (2006) has proposed the *S. arcticum* Geographic Distribution/Taxon-Age hypothesis. Accordingly, cytospecies with broad, geographic distributions may be evolutionarily old and would be expected to be more reproductively isolated in sympatry than presumably younger cytotypes which have more restricted distributions. Given that no large-scale reproductive isolation test had been preformed between *S. arcticum s. s.* and *S. apricarium*, and since LPPC offered the opportunity for such studies we hypothesized that the two cytospecies, *S. arcticum* s. s. and *S. apricarium* would be reproductively isolated at LPPC. We used conventional methods of collection and cytogenetic analysis to study 1108 larvae of the *Simulium arcticum* complex at four sites including the original one and found that *S. apricarium* did not increase in frequency as we sampled down stream. In fact, *S. apricarium* was dominant only in one of 15 collections. Therefore, we reject my first hypothesis. Moreover, we found little evidence for hybridization between the two cytospecies and this observation supports the Geographic Distribution/Taxon-Age hypothesis.

SPECIATION IN THE BLACK FLY, *Simulium arcticum* Complex (DIPTERA: SIMULIIDAE). **Gerald F. Shields** Carroll College, Helena, MT.

Nine sibling species and at least 12 additional cytotypes have been described within the *Simulium arcticum* complex. Sixteen of these taxa occur in western Montana. Such considerable diversity within a localized setting stimulates hypotheses about the mechanisms responsible. Fortunately, there is considerable information on geographic distributions of various taxa within the *S. arcticum* complex and these in conjunction with the cytogenetic data can be used to formulate models about the processes underlying diversification. Correspondingly, I present the *S. arcticum* "*Geographic Distribution / Taxon Age*" Hypothesis. Accordingly, most siblings have broad, continent-wide distributions while cytotypes have very limited, local distributions, some being found at only a single collection site. If the assumption is made that siblings are old in an evolutionary sense and cytotypes are correspondingly young, then the former might be reproductively isolated from one another in sympatry while the latter may not be. In addition to describing diversity within the complex, our group has recently focused on two research themes: 1) study of environmental determinants of taxon distribution and 2) assessment of the reproductive status of cytospecies and cytotypes in sympatry. For the latter research focus we have evidence that siblings (cytospecies) in sympatry are reproductively isolated while cytotypes are not. These observations indicate that sex-linked chromosomal inversions play some role in reproductive isolation within the *S. arcticum* complex and continued similar tests of this hypothesis may lead to a model for speciation within this group.

The *Simulium arcticum* Complex: Environmental Effects on Distribution of Taxa at Trout Creek and Reproductive Status of Taxa at the Blackfoot River. **Lindee M. Strizich** and Gerald F. Shields Carroll College, Helena, MT.

REPRODUCTIVE STATUS OF CYTOTYPES OF THE *Simulium arcticum* Complex AT ROCK CREEK, MISSOULA COUNTY, MONTANA. **Judith A. Pickens** and Gerald F. Shields Carroll College, Helena, MT.

Shields (2006) has proposed the *S. arcticum* geographic distribution/taxon age hypothesis in which siblings may be evolutionarily old and cytotypes may be correspondingly young. If so, siblings occurring in sympatry should be reproductively isolated while cytotypes may not be. We tested this hypothesis at Rock Creek, Missoula County, Montana. Analysis of larvae from this site in previous years indicated that: 1) two cytotypes, *S. arcticum* IIL-9 and IIL-19 were dominant, 2) the autosomal polymorphisms, IS-1 and IL-1, were abundant enough to allow equilibrium frequencies to be calculated, 3) the polytene chromosomes of these taxa were of exceptional quality and 4) larvae were very abundant in early March. Analysis of the distributions of the autosomal polymorphisms among more than 500 larvae from a single collection on March 14, 2006 indicated that the population of IIL-9 and IIL-19 was in genetic equilibrium. As hypothesized, the two cytotypes are not reproductively isolated while in sympatry and this observation supports the geographic distribution/taxon age hypothesis.

Mike Spironello Award:

BLACK FLY FAUNA OF NEPAL. **Dustin A. Swanson**, Peter H. AdlerClemson University, Clemson, SC.

Nepal, and the Himalayan Range in general, represents one of the final frontiers of black fly taxonomy. To date, only 10 nominal species of black flies have been recorded from Nepal. Many species likely remain to be discovered. We examined pupae and adults collected in 1992 from Nepal by G.W. Courtney. Three species, *Simulium bagmaticum*, *Simulium novigracile*, and *Simulium griseifrons*, have been identified, the latter two being new records for Nepal. Another species has been identified to the *Simulium multistriatum* species group near *Simulium novolineatum,* which was previously unrecorded in Nepal. Identifications are continuing. To date, all species are in the genus *Simulium*, and the fauna consists of both Oriental and Palearctic elements.

UTILITY OF THE CYTOCHROME OXIDASE I GENE FOR SPECIES RECOGNITION AND PHYLOGEOGRAPHIC ANALYSIS IN BLACK FLIES (DIPTERA: SIMULIIDAE). **Julio Rivera[1],** Douglas C. Currie[1, 2], [1]University of Toronto, Toronto, ON, [2] Royal Ontario Museum, Toronto, ON.

The mitochondrial gene Cytochrome Oxidase I (COI) has recently gained prominence as a tool for species identification. In this study, we investigate the utility of this gene to (a) assess the species status of the widespread cordilleran species *Prosimulium travisi* Stone,

1952; and (b) assess phylogeographic patterns among 55 populations sampled across the entire range of the species. Preliminary results confirm previous cytological evidence that populations of "*P. travisi*" from high altitude habitats in Colorado constitute a separate species. Analysis of population structure suggests that the present day distribution of *P. travisi* was derived from two different refugial areas during the Wisconsinan Glaciation: one northern (Beringia) and one southern. Following deglaciation these founding populations dispersed southward and northward, respectively, and are now sympatric in the region straddling the 49th parallel. Our results show that the COI gene has utility for both species recognition and revealing phylogeographic patterns in black flies.

EFFECTS OF TEMPERATURE AND DEVELOPMENTAL STAGE ON NITROGEN AND CARBON STABLE ISOTOPES IN THE BLACK FLY, *Simulium vittatum* IS-7. **Grant Howell,** Jay Overmyer**,** University of Georgia, Athens.

The effects of water temperature and developmental stage of the black fly, *Simulium vittatum* cytospecies IS-7, on nitrogen (^{15}N) and carbon (^{13}C) stable isotopes were investigated under controlled laboratory conditions. Preliminary results indicate that _^{15}N signatures of the larvae decrease as larval instar increases. These results contradict the theory that ^{15}N accumulates in organisms over time. Pupae and adults also appear to have higher ^{15}N signatures than larvae. No obvious patterns were observed with ^{13}C signatures. As water temperature increased larvae showed an increase in ^{15}N. This also contradicts previously published work in other organisms. Additional repetitions are needed to determine if these relationships are significant.

GAA MOLECULAR APPROACH TO IDENTIFYING MEMBERS OF THE *Simulium jenningsi* SPECIES-GROUP. **Beth Alexander,** University of Tennessee, Knoxville, TN.

Black flies (Simuliidae) in the *Simulium jenningsi* species-group are nuisance pests that require a molecular diagnostic approach to reliably identify females due to uniformity of morphological characters. This approach can then be used to determine host preference and pest status in turn allowing for effective suppression.

VESICULAR STOMATITUS: A RESEARCH PROSPECTUS; STUDY OF DISEASE CYCLE, VECTOR TRANSMISSION, AND VIRUS MAINTENANCE. **Paul Smith,** Danny Mead, Ray Noblet**,** University of Georgia, Athens, GA.

Vesicular stomatitis, a viral disease of livestock, is of great economic importance because detection of the virus in a herd results in quarantine. Previous research has shown that the black fly *Simulium vittatum* can serve as a vector of this disease. The goal of our research is to further evaluate the contribution of insect vectors to transmission and maintenance of disease. We intend to evaluate vertical transmission of the disease within the black fly vector, potential mechanical transmission of the virus by flies, vector saliva impacts on disease transmission, and virus strain impacts on livestock hosts.

Guest Presentation:

SMALL IS SIGNIFICANT, BE IT ORGANISM OR ECOSYSTEM. **Judy Meyer,** University of Georgia.

Abstract not available

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Attendants to the 5th NABFA Meeting

BLACK FLY SWAT TEAM

NABFA 2007

NABFA acknowledges all the attendants for another successful meeting and encourages all members of our association to keep assisting to future meetings.