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The 3rd Annual Meeting of the North American Black Fly Association

Chair: Jay Overmyer
Secretary: Mike Spironello

Talk Titles With Abstracts

Sunday, February 6th

Yes Virginia, we already know there are black flies in Vanuatu. **Doug Craig**

Feminization of larval black flies (Diptera: Simuliidae) infected with mermithid nematodes (Nematoda: Mermithidae) **Amy Sharp** and Fiona F. Hunter, Brock University

Abstract. Feminization is observed in nematode-infected (Nematoda: Mermithidae) larval, pupal and adult black flies (Diptera: Simuliidae). To determine the developmental stage at which feminization occurs, nematode-infected larvae were sexed by examining gonad development and morphology. 40% of the infected larvae had no gonads present at all, 37% had two testes present, 3% had two ovaries present, 7% had one teste and one ovary present, and 13% had only one gonad present. Chromosomal examination will confirm these morphological observations.

Pennsylvania black fly suppression program control problems caused by high stream flow. **Doug Orr**, PA Department of Environmental Protection Black Fly Suppression Program

The Caucasus: a hotspot for black fly symbiotes? **Mark P. Nelder**, Peter H. Adler, Clemson University and Eugenie A. Kachvoryan, Institute of Molecular Biology, National Academy of Science, Yerevan, Armenia.

Abstract. The larvae of 22 species of black flies, including 7 endemic to the Caucasus, were collected from 35 sites in Armenia, from 1998 to 2004, and examined microscopically for gut-inhabiting trichomycete fungi and ichthyosporean protists. We tested the hypothesis that endemism of black flies in the Caucasus of Armenia is reflected in their gut symbiotes. All gut symbiotes, including eight species of trichomycete fungi and two species of ichthyosporeans, were widely distributed, indicating an absence of endemic trichomycetes in Armenian black flies. The lack of host specificity of trichomycetes that colonize larval black flies probably does not constrain the geographic distributions of trichomycetes.

Good species behaving badly: apparent paraphyly of black fly sibling species in the *Simulium arcticum* complex (Diptera: Simuliidae), **Mike Spironello** and Doug Currie, University of Toronto and Royal Ontario Museum

Abstract. Phylogenetic studies showing deviations from species-level monophyly (i.e., paraphyly and polyphyly) have plagued the use of mitochondrial DNA (mtDNA) in elucidating phylogenetic relationships below and above the species-level. What has been overlooked is the potential information that lies within mtDNA gene trees that exhibit nonmonophyletic patterns. In this study, we present genealogical relationships among sibling species in the *Simulium arcticum* species complex using three mitochondrial genes (12S, cyt b, and COII). *S. arcticum* siblings have historically been considered “good species” based on polytene chromosome banding patterns and ecological data. The total-evidence molecular analysis suggested that individual sibling species as currently defined, are paraphyletic. We use the genealogy of *S. arcticum* sibling species to interpret this paraphyly in view of chromosomal and ecological data that indicates cytological characterized entities are indeed “good species”. The advantage of using chromosomal data as a ‘nuclear background’ for elucidating the causes of paraphyly will be discussed.

Public perception of annoyance from black flies in the Twin Cities metropolitan area. **Ken Simmons**, Nancy Read, John Walz, Metropolitan Mosquito Control District, St. Paul, MN

Abstract. The relationship between black fly numbers sampled in overhead sweep nets and the perception of annoyance by the public was examined using simultaneous sampling and surveys. A randomized sample of individuals was asked to stand in their yard for 3 minutes, during which time a staff member took a sweep sample at a location 20 ft. from the participant, and another staff member observed participant defensive behaviors and measured environmental conditions 20 ft from both the participant and the sampler. A total of 274 samples were collected over 2 yrs during times when black flies were active and there were few mosquitoes. Results suggest that black fly levels of 3 or more were related to an apparent increase in annoyance, reduced time outdoors, and interest in wearing repellent. Levels of 10 or more were related to a stronger response. Median reported tolerance for “gnats around you” was 5, for landings was 3, and for bites

was 1. One-third of respondents reported “reacting strongly” to gnat bites. Median amount participants were willing to spend to reduce gnat levels was \$10 overall; those reacting strongly had a median of \$15. Difficulties encountered included relatively few samples with high numbers of black flies, and highly variable results at low black fly levels, similar to those found with mosquitoes (Read, Rooker and Gathman, 1994, J. Am. Mosq. Cont. Assn. 10:79-87). Observed behavior counts did not appear to be closely related to black fly counts or annoyance in this data set.

Black fly biodiversity and speciation from an ecological perspective. **Murray H. Colbo**, Department of Biology, Memorial University, St. John's NL

Abstract. The paper explored the geographical patterns of black fly species richness at different scales and found in North America for example Alaska, NWT were equally or more species rich to many comparable sized southern areas. The ecological characteristics related to these geographic patterns were examined. One pattern found suggested cool water habitats are more diverse. Also it is evident that the evolution of the female fly behaviour is critical to species diversity as she selects the larval habitat, but this is an aspect of black fly biology that is poorly known.

Spatial & temporal heterogeneity: implications for the ecology of low-gradient streams, **Len Smock**, Department of Biology, Virginia Commonwealth University

Post-translation modifications and physical characterization of larval salivary gland proteins, **Andrius Masedunskas**, Lewis Pannell and Charles Brockhouse, University of South Alabama

Abstract. Black flies are one of the few groups of insects that secrete an aquatic silk. Silks have long attracted attention as biodegradable fibers of considerable strength elasticity and durability. Unlike aquatic, terrestrial silks are well characterized, particularly silkworm and spider silks. Black fly larvae produce large quantities of glue-like silk, which is used for anchorage to the substrate in rapid currents, resettlement and production of pupal cocoon. Two major salivary gland proteins (sgps) have been previously found in salivary glands of genus *Simulium* larvae: a sgp with a MW of approximately 40,000 daltons and a second type of sgp varying in size from 65,000 to 115,000 daltons. The exact size of proteins in each class depends on species and even sibling species (Brockhouse and Tanguay, 1996. J. Agric. Entomol 13(4), pp. 339-347). When treated as alleles different MW sgps can provide a valuable tool for examining the population structure. SDS-PAGE (PolyAcrylamide Gel Electrophoresis) assay was used to examine and compare the populations of *Simulium ubiquitum* (wild) and *Simulium vittatum* Zetterstedt sibling species IS-7 (colony sustained in laboratory settings since 1981). Heterozygosity and three size variants in higher MW class sgp were detected revealing a polymorphic character of the *S. ubiquitum* population. Lack of sgps size variation within *S. vittatum* population implied that the population is monomorphic for the allele. Another part of the study examined posttranslational modifications of silk

proteins using glycosylation and phosphorylation staining, two-dimensional gel electrophoresis (2DE) and mass spectrometry (MS). Evidence for posttranslational modifications suggested that of both sgps were highly phosphorylated. Higher MW class salivary gland protein seemed to be slightly glycosylated.

Black fly involvement in the epidemic transmission of vesicular stomatitis New Jersey virus. **Daniel Mead**, Southeastern Cooperative Wildlife Disease Study, College of Veterinary Medicine, University of Georgia.

Abstract. *Vesicular stomatitis New Jersey virus* (VSNJV) is a causative agent of vesicular stomatitis (VS), an Office International des Epizooties List-A disease. VS is an economically important disease which primarily affects cattle, swine, and horses. Accumulating field and laboratory evidence supports the thesis that insects, including black flies, are involved in epidemic VSNJV transmission. However, because a viremia has never been detected in any domestic animal species following natural or experimental VSNJV infection, skepticism remains regarding the role of insects in VSNJV transmission during epidemics since the mechanisms by which they become infected is unknown. In recent experiments we have demonstrated that black flies (*Simulium vittatum*) are capable of transmitting the virus to domestic pigs and horses and that the development of clinical VS is bite site dependent. Clinical VS developed only when VSNJV infected black flies fed on the snout (pigs) or muzzle (horses). In addition, we have demonstrated that black flies can become infected with VSNJV by feeding on virus rich lesions and by co-feeding. These data significantly add to our understanding of the transmission routes of a causative agent of one of the oldest known infectious diseases of livestock, for which the details have remained largely unknown despite decades of research.

Black fly control in the urban west: problems and solutions at the greater Los Angeles County vector control district. **Paul F. O'Connor**, Jack E. Hazelrigg, Michael W. Shaw and Minoo B. Madon, Greater Los Angeles County Vector Control District

Sibling species of the black fly, *Simulium arcticum* complex, Diptera: Simuliidae emerge at different elevations. **Gerald F. Shields**, Christina S. Marchion, Tracy L. Michel, Kathryn C. Styren, Callie N. Riggins and Tonya D. Santoro, Department of Natural Sciences, Carroll College

Abstract. We used conventional methods of collection and cytogenetic analysis to study 1432 larvae of the *Simulium arcticum* complex at various elevations within four separate drainages in western Montana to test the hypothesis that the northwesterly distributed *S. arcticum sensu stricto* would occur at high elevations and the southeasterly distributed *S. apricarium* would occur at low elevations. This research attempts to identify reasons for the distributions of various sibling species of the *S. arcticum* complex within an apparent contact zone between the two siblings. Two of the four drainages tested, the Boulder River and Canyon Creek/ Little Prickly Pear Creek, flow south and east and eventually

drain into the Missouri River. The other two drainages, Flint Creek and the Little Blackfoot River, flow north and west into the Clark Fork River that drains northwestern Montana. With a single exception, *S. apricarium* was absent from all high elevation sites and from three of the four intermediate elevation sites; alternatively, it was the only member of the complex at one low elevation site, abundant at a second, and present at the other two. *S. arcticum s. s.* was abundant at all high and intermediate elevation sites. It was absent or rare at two low elevation sites, infrequent at a third but abundant at a fourth. Contrary to the ratios at the other nine sites, *S. arcticum s. s.* was four times more abundant than *S. apricarium* at Little Prickly Pear Creek, a low elevation site. We are currently investigating hypotheses about the sibling ratio at this site and this drainage.

More on sibling species diversity within the *Simulium arcticum* complex, Diptera: Simuliidae in Montana. **Gerald F. Shields**, Department of Natural Sciences, Carroll College

Abstract. In a continuing effort to investigate the apparent widespread diversity of sibling species within the *S. arcticum* complex in Montana and the Pacific Northwest and based on more than 500 site collections, from 35 drainages and more than 3,300 larvae analyzed chromosomally, we have discovered the presence of five sibling species (*S. brevicercum*, *S. saxosum*, *S. arcticum s.s.*, *S. apricarium*, and *S. negativum*) all of which are abundant and widespread geographically. Additionally, 10 cytotypes, each having unique paracentric inversions associated with sex (IIL-9, IIL-10, IIL-13, IIL-15, IIL-17, IIL-18, IIL-21, IIL-22, IIL-23.24.25, and IIS-12) have been discovered. With the exception of IIL-15 and IIL-18, these tend to be infrequent and locally distributed. Most of these cytotypes have some characteristics (separate emergences from other types, overwintering as larvae, presence of B chromosomes, unique autosomal inversions) that suggest that some or all of them might become real siblings when their reproductive status is investigated further. These populations which frequently: exhibit sex-chromosome polymorphisms in linkage disequilibria, share extensive autosomal polymorphisms but unique derived types, and possess some differences in their biology and present-day distributions suggest that a sympatric mode of speciation may be occurring. Future efforts will expand the geographic region studied, determine the reproductive status of cytotypes in sympatry, and attempt to determine the causal factors associated with distribution of types.

Experiences in black fly research program in the region of Novi Sad (Serbia and Montenegro) and control by application of B.t.i. **Aleksandra Ignjatovic Cupina**, Dusan Petric, Aleksandra Konjevic, Marija Zgomba, and Dusan Marinkovic University of Novi Sad, Serbia and Montenegro

Abstract. The elevated biting activity of black flies was recorded in the region during last few years. Research program of black fly fauna based on permanent monitoring of populations in adult and immature stages has been intensified starting from 2001.

Among nine identified species *S. ornatum* (complex) was dominant and permanently present species, with four recognized overlapping generations/year and the highest anthropophilic preference. Abundant breeding sites were located in majority of streams of the Fruska Gora hills.

Larvicide control measures of black flies have never been applied in the country before. Treatments of the second generation of *S. ornatum* (complex) larvae by application of B.t.i. (VectoBac 12AS) in two different concentrations (5 ppm and 25 ppm) were performed in 2004. Population density was estimated before and after the treatments in control points by collecting samples of immature stages on submerged vegetation (twigs in length 3x10cm), daily and weekly attachment rate on plastic strips (30cm x 4cm).

Application of two different concentrations did not confirm significant differences in larval mortality. In both cases total reduction (100%) has been recorded within the stream section of 450 to 500 meters in length. Exceeding the distance from the treatment point the number of survived larvae increased.

Just a little southeast of Nome: the black flies of East Beringia, **Peter H. Adler**, Clemson University and Doug C. Currie, University of Toronto and Royal Ontario Museum

Distribution, life history and pest status of the *Simulium jenningsi* species group in Pennsylvania, **Dave Rebutck**, Pennsylvania Department of Environmental Protection-Division of Vector Management.

Alimentary System of Larval Black Flies (Diptera: Simuliidae): Structure and Phylogeny **Samkyu KIM** and Peter H. Adler, Department of Entomology, Soils and Plant Sciences, Clemson University

Abstract. Gut structures of 15 genera, including 14 subgenera, and 33 species of larval black flies (Diptera: Simuliidae) were examined and used in cladistic analysis to infer their phylogeny for the first time. Among the families of Superfamily Chironomoidea, the family Thaumaleidae was used as outgroup. Cladogram inferred from 13 characters from gut structures including 3 characters from foregut spine structure of larval black flies contained many unexpected relationships. In addition, possible functions of foregut spines in larval black flies were also discussed.

A genetic look at the black flies of the Galapagos, **Gillian Richard**, Charles Brockhouse, University of South Alabama, Cecilia Coscaron-Arias, Darwin Foundation

Abstract. The black fly, *Simulium ochraceum s.l.*, is believed to have reached San Cristobal Island, Galapagos prior to 1989, when reports of black fly biting began. The focus of this study is to establish the population structure of *Simulium ochraceum s.l.* on San Cristobal Island in order to determine if there has been a single or multiple introductions. We approached this problem by measuring the degree of heterozygosity and distribution in the collected samples. Enzymatic methods were used as a means of

indirectly resolving genetic diversity among individuals and/or sites. The results denoted the possible presence of one or more polymorphisms in 60-70% of the individuals tested. As a more direct analysis, DNA from various individuals was sequenced and the results indicated polymorphisms in the majority of the individuals, many of which contained multiple polymorphic sites. Together, these results suggest either multiple introductions or a long-standing presence on the island.

Notes on trichomycetes: development, growth and a few new tricks, **Svjetlana Vojvodic**, John McCreadie, University of South Alabama

Monday, February 7th

Barcodes for black flies: can COI sequence data accurately distinguish species? **Doug Currie**, University of Toronto and Royal Ontario Museum and Peter Adler, Clemson University

Abstract. “DNA taxonomy” and “DNA barcoding” have attracted considerable attention as an alternative means to identify species. We present the first test case of the utility of the COI gene for distinguishing species of black flies. Two hundred and thirty nine samples representing 5 genera and 24 species were analyzed, and the resulting neighbor-joining tree accurately resolved 18 of 24 species. Problematic samples were sibling species in the *Simulium venustum*- and *Simulium tuberosum* species complexes. Such cryptic species evidently diverged relatively recently from each other, and it is not yet clear whether a different gene (or a combination of genes) can accurately distinguish them. These mixed results notwithstanding, the COI gene showed sufficient promise to extend this study to additional species and genera of simuliids. Black flies are notorious for their structural homogeneity and not all life-history stages are equally amenable to morphological- or cytological identification. Development of a DNA based identification system would give taxonomists another tool to deal with hitherto intractable life-stages of particular taxa.

Landscape features and species assemblages - the big, big picture, **John McCreadie**, University of South Alabama, and Peter Adler, Clemson University

Molecular phylogeny of *Cnephia*, **Kenneth Pruess**

Abstract. Five species of *Cnephia* were sequenced for the complete mitochondrial CO-I gene and portions of nuclear EF1-alpha and dopadecarboxylase genes. Resultant phylogenies all supported *Cnephia* as a monophyletic clade within a monophyletic Simuliini. Sequences were not completely concordant but generally supported *C. ornithophilia* and *C. pallipes* as having longer evolutionary histories. Molecular diversity in *C. ornithophilia* was greatest in southern US, decreasing to the north. *C. pecuarum* also

represents an early change from bird to mammal feeding. Non-feeding *C. eremites* and *C. dacotensis* are derived. There was little difference in any sequences between Canadian and Swedish *C. eremites*. Additional specimens of *Cnephia* in alcohol are solicited.

West Virginia Department of Agriculture Black Fly Control Program, **Lois E. Swoboda**,
West Virginia Department of Agriculture

Mitigating the effects of algae on *B.t.i* efficacy: exposure concentration and time, **Jay Overmyer**, Elmer Gray, and Ray Noblet, University of Georgia

Abstract. It has been documented in several black fly control programs around the world that when algal populations are elevated in rivers, the efficacy of *Bacillus thuringiensis* var. *israelensis* (*B.t.i*) is reduced. However, little is known about which types of algae play a role in this problem or what methods can be implemented to mitigate these effects. In this study, four species of algae commonly present in rivers when efficacy is reduced, *Microcystis* spp., *Dictyosphaerium* spp., *Chlorella* spp. and *Scenedesmus* spp., were screened in an orbital shaker bioassay to determine which species were inhibiting efficacy. Of the four, *Scenedesmus* spp. was the only species that significantly reduced mortality in black flies exposed to *B.t.i*. Additional experiments were conducted with the orbital shaker bioassay to determine what actions could be implemented to mitigate the effects of *Scenedesmus* spp. Increasing the dose of *B.t.i*. applied to the flask proved to be effective as well as increasing exposure time of the larvae to the insecticide. Further analysis of black fly mortality over time indicates that the toxicity of *B.t.i* is not inhibited. However, the mechanism by which *Scenedesmus* spp. affects efficacy causes mortality to be elicited approximately 2 to 3 times slower than when no algae is present. Experiments are currently being conducted to determine the mechanisms associated with this process.

Pasco County, FL update

Black flies, tanning, and health concerns **Eddie Beard**, Clemson University

Abstract. We are starting an investigation of black flies and their symbiotes in southeastern Coastal Plain streams. Tannic acid is a common phenolic in these streams and it might have consequences for black fly biology. We have a special interest in tannins because they precipitate and complex with proteins, but tannins are a large group of chemicals and difficult to assay consistently in a stream environment. Tannins and phenolics have not been included in most stream characterizations of black fly ecology studies. Affects attributed to tannins in other studies with Diptera include; death associated with midgut lesions, slower development, and increased virulence of ciliate pathogens. Another consideration is the potential effect on Bti toxins since they are proteins and efficacy can be impacted. The biology of black fly symbiotes such as trichomycete fungi might be affected by tannin content. We ask whether tannins can

continue to be ignored in studies that characterize the habitats of black flies, especially in blackwater streams.