

Recurring Late December Outbreaks of Adult *Simulium jenningsi* Group Black Flies (Diptera: Simuliidae) in Seminary, Mississippi, USA¹

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Abstract Black flies (Diptera: Simuliidae) are reemerging as biting and nuisance pests in many southern states, presumably from improving water quality in creeks and rivers. Since 2009, entomologists at Mississippi State University and the Mississippi Department of Health have conducted surveys to ascertain what black fly species are present in the state as well as their geographic distribution and seasonality. These surveys revealed what appears to be a recurring, significant emergence of black flies every year around 25 December at one site in southern Mississippi. In this study, adult black flies were collected from 1 January 2018 to 31 December 2021 by hand netting in the exact same way each time at Okatoma Creek, Seminary, MS. Forty-eight collecting trips to the site over the 4-yr period yielded a total of 176 black flies, all morphologically identified as *Simulium jenningsi* Group Malloch. Molecular identification was successfully performed on 17 specimens collected during the December outbreaks. Of the 17 specimens analyzed, 10 and 7 specimens grouped with 100% bootstrap confidence inside clades comprising *S. jenningsi* or *S. podostemi*, respectively.

Key Words black flies, emergence, seasonality, outbreaks, Mississippi

Some of the first well-documented descriptions of black fly outbreaks in the southern United States were from Greenville, MS, and Clarendon, AR, in 1859 (Webster 1887, 1904). Beginning in the late 1920s and early 1930s, many reports and complaints were received from Mississippi county extension agents, veterinarians, physicians, and farmers about “gnat” attacks. These pests were reported as *Cnephia pecuarum* (Riley) and *Simulium meridionale* Riley, and they prompted Dr. George Bradley’s extensive research on black flies, focusing primarily on the Mississippi Delta (Nations et al. 2016). However, black fly problems dissipated in Mississippi for the next 8 decades. In 2008–2009, the Mississippi State Department of Health and the Mississippi State University Extension Service began receiving complaints from the public about increased human biting incidents and backyard poultry deaths resulting from black flies.

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As a result, researchers from Mississippi State University began systematic collections of black flies from >20 sites around the state, resulting in several published works demonstrating two predominant species occurring in Mississippi—*S. jenningsi* Group Malloch and *S. meridionale* (Goddard and Nations 2021; Jones et al. 2014; Nations et al. 2018, 2020). As had been previously reported in other states (Amrine 1982, Voshell and Reese 1991), black flies in the *S. jenningsi* Group were found to be multivoltine in Mississippi, producing several generations from spring until fall. However, our surveys revealed what appears to be a recurring, significant emergence of black flies every year around 25 December (third week of December) at a site near Seminary, MS. This study was an attempt to investigate this phenomenon and ascertain the species involved.

Materials and Methods

Black fly collections. Adult black flies were collected from 1 January 2018 to 31 December 2021 by using a 1-m standard “student” insect net with a 30-cm net opening (BioQuip Inc., Rancho Dominguez, CA). Upon each visit, the net was swept overhead in a “figure 8” configuration two to three times and then checked for black flies. This sweeping was repeated over and over for a total time of 10 min. All collections were performed in the exact same way and place each time, all within 20 m of Okatoma Creek, Seminary, MS. The site is approximately 160 km north of the Mississippi Gulf Coast. Collections were made monthly, although only 10 collections were made during 2020 due to the severe acute respiratory syndrome coronavirus 2 pandemic. All collected specimens were placed in 70% ethanol and transported to the laboratory for identification by using published keys (Adler et al. 2004, Stone and Snoddy 1969). Subsamples of each species were sent to Dr. Peter Adler (Clemson University, Clemson, SC) for confirmation. Voucher specimens are deposited in the Mississippi State University Entomological Museum.

Molecular identification. Seventeen black fly specimens, stored in 70% ethanol, that had been collected during the third week of December (2018–2021) were submitted to the second author (JKM) for molecular identification by using methods described previously (Senatore et al. 2014). In brief, individual specimens were lysed overnight by using a sodium dodecyl sulfate–based buffer including Proteinase K, and DNA was extracted and purified using silica columns. DNA was amplified via polymerase chain reaction by using the overlapping primer combinations 171F (5′-GTNGGNTGGGGNAARAARGARAC-3′) with SJG P1R (5′-ACGATTTCDATCTTRTCACA-3′) and SJG P2F (5′-TTRCAAGCGGTGATGAYCA-3′) with 959R (5′-AANGGNAGRTAYTCYTTNGGRTCYTT-3′) and the cycling parameters describing in Senatore et al. (2014). Sanger sequencing procedures differed from those presented in Senatore et al. (2014) in that Big Dye 3.1 dye terminators (Applied Biosystems, Waltham, MA) were substituted with QuantamSeq 3.1 dye terminators (SureFire Biosciences, San Diego, CA), and reaction volume was reduced to 15 µl. After reconciliation of generated sequences by using Sequencher 5.4.6 (Gene Codes Corp., Ann Arbor, MI) and excision of priming regions, the two pieces were combined into a single fragment of 1,999 nucleotides. Resulting fragments from the 17 individuals tested were added to a data matrix containing the Senatore et al. (2014) data and then analyzed

Table 1. Results of molecular identification of 17 *Simulium* specimens collected in the third week of December, 2018–2021, Seminary, MS.

Species	2018	2019	2020	2021
<i>S. jenningsi</i> s.s.	1F	4F	3F	2F
<i>S. podostemi</i>	1F	1F	2F	3F

F = female.

phylogenetically using IQ-TREE, Version 2.2.0. We inferred the maximum-likelihood tree by using the edge-linked partition model in IQ-TREE (Nguyen et al. 2014, Chernomor et al. 2016). Node support was assessed using the ultrafast bootstrap method (Hoang et al. 2018). Representative sequences of each observed genotype are deposited in GenBank under accessions OP264081 and OP264082.

Results and Discussion

Forty-eight collecting trips to the Okatoma Creek over the 4-yr period yielded a total of 176 black flies, all morphologically identified as *S. jenningsi* Group members. Eighteen (37.5%) of the 48 trips yielded no specimens. Although we assume these black flies all originated from Okatoma Creek because they were collected within sight of the water, we found no presence of black fly larvae or pupae in the creek itself, despite three attempts during various times of the year. Members of the *S. jenningsi* Group are notoriously strong fliers, so it is possible they could be using the drainageway as a flight corridor from an adjoining water source.

DNA was successfully extracted and sequenced from 17 specimens collected during the December outbreaks and preserved in 70% ethanol. Of the 17 specimens analyzed, 10 and 7 specimens grouped with 100% bootstrap confidence inside clades comprising *S. jenningsi* or *S. podostemi* Snoddy, respectively (Table 1). *Simulium podostemi* is a medium-to-large river species known from Pennsylvania to Mississippi and Arkansas. It has been previously collected in northern Mississippi. Members of the *S. jenningsi* group (~22 species) are found in eastern North America, generally in streams and rivers greater than 6 m in width (Adler et al. 2004, Amrine 1982, Moulton and Adler 1995). Okatoma Creek at Seminary is approximately 7 m in width.

In our study, black flies were collected each month year-round except for January (although we did collect in January), with a huge peak during the third week of December (Fig. 1). Three times during the 4-yr study, black flies were collected at an extrapolated rate of nearly 100 individuals/h, each time within 3 d of 25 December. In no other month during any of the 4-yr period were black flies collected at this rate. Other researchers have reported multiple overlapping generations of *S. jenningsi* Group per year, leading to a near continuous emergence of adults between midspring and late fall (Voshell and Reese 1991), but none lasting until late December. Members of the *S. jenningsi* Group are known to be pollution intolerant; thus, increasing numbers and range expansion may be due to improved

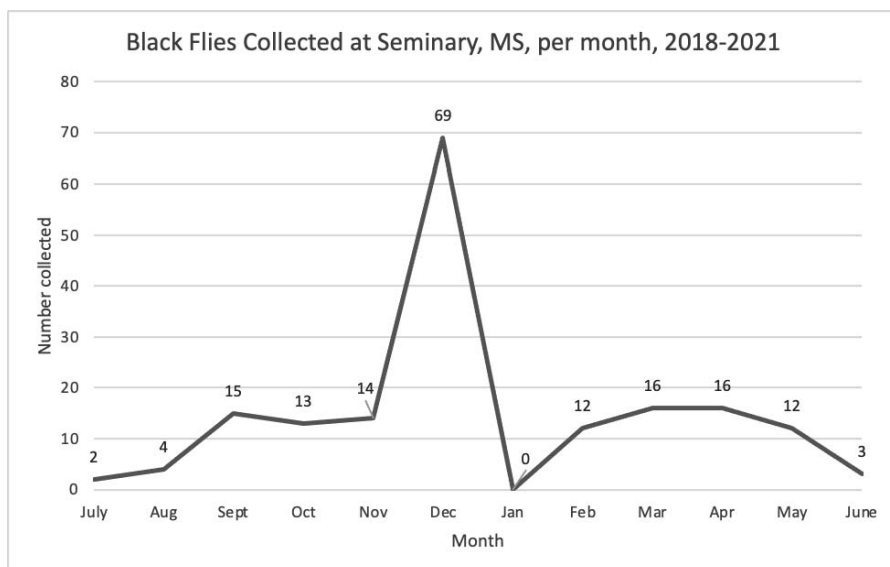


Fig. 1. Seasonal distribution of black flies collected at Seminary, MS, monthly numbers combined, 2018–2021.

water quality (Gaudreau and Charpentier 2011). However, the outbreaks reported herein may be due to long-term, late-year warming trends due to climate change.

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