

H19 Geographic, Landscape, and Sex Variation in Adult Blow Fly Microbiomes: Implications for Forensics

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Learning Overview: After attending this presentation, attendees will better understand how geographic and landscape (i.e., urban and rural) differences affect the microbiomes of adult male and female blow flies (Diptera: Calliphoridae). Attendees will also be introduced to the potential importance of blow fly geographic variation and their associated microbiomes within the broader field of forensic science.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by providing information on the geographic distribution and diversity of forensically relevant blow communities with their nested internal microbiomes. This presentation will provide new data on how adult fly microbiomes vary between sexes captured in three geographic locations of the United States (Appalachia, southeast Alaska, and Mid-Michigan ecoregions), including an analysis of the differences in microbiome structure for blow flies collected in rural versus urban landscapes in Mid-Michigan. Blow flies are forensically important insects used to help estimate a Postmortem Interval (PMI), as they are often the first species to locate and colonize decomposing bodies, including humans.¹ Previous research has shown that microbiomes of flies may affect the succession of insect communities on carrion.² The microorganisms influence the decomposition of organic matter by mediating ecological interactions that exist during decomposition.²

It was hypothesized (H1) that geographical location and urban versus rural environments of adult blow flies would affect blow fly communities and their internal microbiomes. Blow fly microbiomes are influenced by their environment, and distinct populations of blow flies exist across United States geographic regions, as determined using molecular markers.^{3,4} Thus, this variation in genetic populations led to the assumption that blow fly microbiomes could also be distinct based on population, in which ecoregion was used as a proxy for populations. It was further hypothesized (H2) that male and female microbiomes at site-specific locations would vary because the sexes have different behaviors associated with colonizing carrion (e.g., females have extensive contact and lay eggs, but males do not).

To test these hypotheses, blow flies were collected using passive bait traps from nine sites within six ecoregions of the eastern United States (henceforth Appalachia), seven sites near Juneau, AK, and seven sites in Mid-Michigan.⁵ Within the Mid-Michigan locations, blow flies were captured along an urban to rural gradient. Urban locations were located in landscapes predominantly developed, whereas rural locations had low development and an abundance of open land.⁶ After collection, DNA was extracted using a magnetic bead-based protocol, and the internal fly bacterial communities were identified using high-throughput targeted amplicon sequencing of the 16S gene (V4 region) using methods from published studies.⁷⁻⁹ Of the blow flies collected in Appalachia, 90.9% were *Phormia regina* (Meigen) ($n = 11,052$) and *P. regina* accounted for 76.9% of the population in rural locations in Mid-Michigan. *Calliphora terraenovae* (Robineau-Desvoidy) was the most abundant species collected in Alaska, accounting for 60% ($n = 160$) of the blow fly communities. *Lucilia sericata* (Meigen) was more abundant (27.4%) in urban areas than rural areas in Mid-Michigan. The internal microbiomes varied among locations and sex of blow flies. From the Appalachia locations, the three predominant phyla detected were Bacteroidetes, Firmicutes, and Proteobacteria. There were significant differences between internal microbiomes of the sexes, with female microbiomes represented by all three phyla and males primarily by Bacteroidetes. The internal microbiomes of female blow flies collected from Alaska consisted predominantly of Proteobacteria and Firmicutes.

Overall, the internal microbial community structures changed depending on the geographical location of adult blow flies. Microbiome differences between male and females also varied significantly. Differences between male and female blow flies resulted in sex-specific microbial indicators, which can be used to further forensic investigations. These data confirm that internal microbiome communities vary between geographic regions and in the future could be used as forensic evidence in criminal investigations. The data could be used to answer questions about the location and environment of the crime scene, as well as estimate PMI. Using microbiomes of forensically relevant blow flies offers ways to improve predicting PMI, which in turn lowers error rates in criminal investigations.¹⁰

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Blow Fly, Microbiome, Geographical Locations