H15 Microbes as the Puppet Master: Clear Evidence Microbes Drive the Decomposition Process and Colonization by Insects

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Learning Overview: After attending this presentation, attendees will know microbes play a key role in driving decomposition of vertebrate remains and the subsequent attraction and colonization by necrophagous insects.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by providing information regarding the mechanisms regulating attraction and colonization of vertebrate remains by blow flies. This information can lead to increased accuracy when estimating forensically important timelines using entomological evidence.

Carrion decomposition typically consists of five stages: fresh, bloated, active decay, advanced decay, and dry. Stages are usually described by microbial and insect activity within and around the remains, and the progression through these stages is thought to be governed by microbial and insect activity in association with other biotic and abiotic factors. Although guided by insect activity, the previously described stages described by Dr. Jerry Payne do not encompass the entirety of the interaction between insects and a resource.[1] The Postmortem Interval (PMI) is defined as the period of time between death and discovery of remains. This is further divided into the pre-colonization interval and post-colonization interval, separated by insect colonization of the remains. Currently, forensic entomologists are capable of calculating the post-colonization interval based on development data available for some necrophagous insects, but the biotic and abiotic factors affecting the pre-colonization interval remain poorly understood and therefore cannot be included in a forensic entomology estimate.

The pre-colonization interval includes the time it takes for insects to detect and locate the resource before colonization. The detection and location phases of the entomological timeline are governed by Volatile Organic Compounds (VOCs) emitted by the remains and associated microbes as a by-product of microbial metabolism. The VOCs produced are responsible for bloat and progression through decomposition and serve as a key mechanism regulating blow fly (Diptera: Calliphoridae) attraction and colonization. However, the degree to which the microbes govern attraction of necrophagous insects is still unknown. Once the factors governing the attraction and location of a resource by necrophagous insects are understood, more accurate forensically relevant estimates can be made.

In the current study, the impact of microbial presence is determined by exposing adult *Cochliomyia macellaria* (Diptera: Calliphoridae) to mouse carcasses with and without microbes in a dual-choice cube olfactometer. Germ-free (axenic) and specific pathogen free (xenic) carcasses were received and preserved overnight at 4°C as preliminary data indicate that freezing a resource alters the attraction of *C. macellaria* to a resource. Axenic and xenic mice were allowed to decompose in a controlled environment to assess the stages of decomposition. A dual-choice cube olfactometer was used to determine preference of adult *C. macellaria* when given the choice of axenic or xenic mouse carcasses based on the VOCs emitted from the treatments. At the conclusion of each trial, all carcasses were exposed to adult flies that were allowed to oviposit on their carcass of choice.

This study determined that the bloat stage of decomposition is absent in axenic mice, as well as later fluid purge. Non-axenic fluid purge occurred on day 6 while axenic fluid purge did not occur until day 13. Olfactometer results indicate that the presence of microbes associated with carrion is a key factor driving attraction of blow flies to carrion at different stages throughout decomposition. In addition, microbial absence reduces *C. macellaria* oviposition by almost 90%.

This study is the first of its kind to completely exclude microbes from the decomposition process and assess the role that microbes play in the progression of decomposition and the attraction of a primary colonizer of carrion. Such data show that microbes are the main driver of attraction and colonization of remains by blow flies and serve as a foundation of exploring microbial variation across decomposing resources in natural settings.

Reference(s):

1. Payne, Jerry A. 1965. A Summer Carrion Study of the Baby Pig *Sus Scrofa* Linnaeus. *Ecology* 46 (5): 592–602. https://doi.org/10.2307/1934999.

Forensic Entomology, Necrobiome, Decomposition Ecology

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