E124 The Identification of Necrophagous Insect Species by Ambient Ionization Mass Spectrometry and Chemometrics for Postmortem Interval Estimations

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Learning Overview: After attending this presentation, attendees will have a greater understanding of the principles of ambient ionization mass spectrometry and its applications to forensic entomology. Additionally, attendees will learn about artificial neural networks and Self-Organizing Maps (SOMs) and how they can be generated from mass spectral data and utilized to determine the species identity of sample unknowns.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by demonstrating the utility of ambient ionization mass spectrometry techniques in forensic entomology. The ability to rapidly analyze and identify necrophagous insects discovered on a corpse, or in animal or elder care abuse cases, will reduce some of the challenges and shortcomings of traditional species identification methods and enable more rapid analysis and species identification.

Conventional methods of species identification of necrophagous insects for determination of the postmortem interval in medicolegal investigations are often time- and resource-consuming. Furthermore, the results can easily be influenced by a number of factors, including the manner in which death occurred, the environment and temperature in the area of the body, and the climate and time of year. Presented here is an alternative mass spectrometric and chemometric approach to species identification of blow flies at each of their life stages (eggs, larvae, pupae, and adults). Application of this approach has revealed: (1) information regarding the biomarkers responsible for the differentiation between species at each life stage, (2) that even closely related species can be differentiated, and (3) that SOMs derived from mass spectral data provide a highly accurate means of identifying insect species.

Egg, larva, pupa, and adult stage samples of blow flies belonging to six species within the order Diptera were collected and suspended in 70% aqueous ethanol storage solutions, which is the conventional practice for storage of entomological evidence discovered on or near a corpse. These solutions were then rapidly analyzed by Direct Analysis in Real Time-High-Resolution Mass Spectrometry (DART®-HRMS) to determine the unique chemical fingerprint spectra. These analyses were performed in replicates of five. The mass spectra were then subjected to chemometric processing in the form of Kohonen SOMs. After initial preprocessing of the raw mass spectral data, minimum Redundancy Maximal Relevance (mRMR) and Sparse Discriminant Analysis (SDA) methods were applied in order to reduce the data to contain only the most significant variables required to create the models. The reduced data were then separated into training and validation sets in order to test the prediction abilities of the models. Mixtures of several species' ethanol suspensions at a given life stage were then created and analyzed to further test the models.

The results indicated that the DART®-HRMS spectral profiles of the blow flies analyzed were highly consistent between replicates but showed interspecies and life stage differences. These fingerprint profiles were then used as the basis for the creation of SOM models, which revealed the mass-to-charge (*m/z*) values of the chemical components that were the most important for discriminating between and identifying different species at each life stage. The cross-validation results for the predictive ability of each life stage SOM were above 93%, and the external validation accuracy was 100% for all models. In the case of the mixture samples, preliminary results indicate 97% accuracy.

The DART®-HRMS chemical fingerprint profiles that were generated were found to be suitable for chemometric processing in order to accomplish species discrimination. DART®-HRMS is a rapid means to rapidly collect unique mass spectra for many different species and life stages of necrophagous insects. The SOM models that were built can be further expanded to include more species and families of insects and create a robust database to assist in the determination of insect species identity and by extension, postmortem interval.

DART®-HRMS, Entomology, Chemometrics