Identification of host characteristics from tick nymphs using mass spectrometric techniques

This work is being done in collaboration with Alan Barbour's lab at the University of California Irvine http://spiro.mmg.uci.edu/index.html

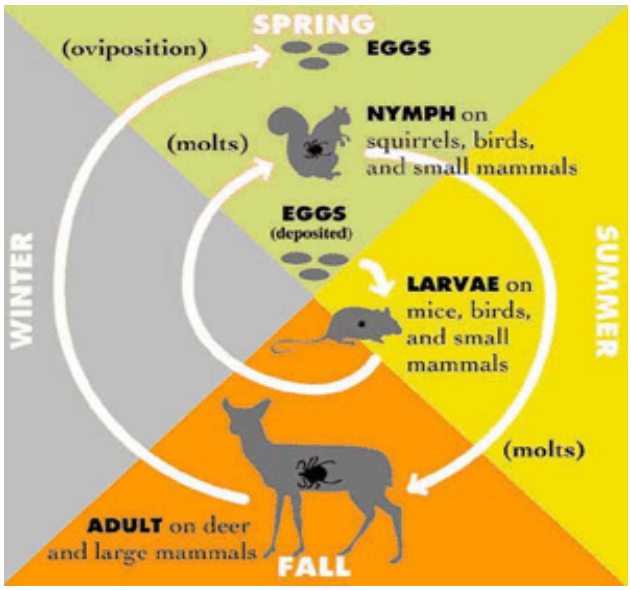
Tick nymphs are vectors that can transfer Lyme disease caused by the bacteria called *Borrelia burgdiferi.* Much research has been done to identify and characterize this disease causing organism but very few chemical studies have been carried out to identify the host that carries this parasite. Mass spectrometry can be used to study these characteristics because of its high sensitivity and selectivity. In this study three different mass spectrometric techniques were used to identify the characteristics of the host that the tick nymphs were fed on. *Amblyoma americanum* tick nymphs were used for these studies which were fed only once with either rabbit or sheep blood three months before the experiments started.

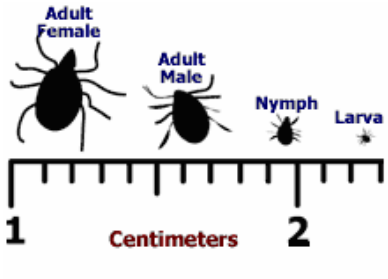
Samples were prepared by pulverizing 15 tick nymphs using a mortar and pestle. The proteins were precipitated with ethanol. Precipitated proteins were separated using 1D SDS PAGE and digested with trypsin. Digested peptides were analyzed by LC-MSMS using a LCQ. SEQUEST database search algorithm was run against a known animal database to identify proteins from the data obtained form LC-MSMS runs. Fatty acids were extracted from the remaining portion of the sample and methyl esters were prepared and analyzed using GC-TOF mass spectrometer. Also a single tick was pulverized, mixed with silicon powder and analyzed by MALDI mass spectrometry using a new ionization method called SPALDI.

Using a 1D gel separation we were able to identify tick proteins along with proteins from the host animal. Among the host animal proteins we were able to identify sheep and rabbit hemoglobin subunits. The tick nymphs we used were fed three months before they came to the lab. This indicates that there is still host information retained in the tick nymphs. This might lead to the identification of the host from a tick nymph found in the forest. In the case of Lyme disease, if the host animal can be identified by the vector we can control the population of the infected host.

It is believed that ticks will retain the lipid portion from the host without digesting it completely. This led us to analyze their fatty acid profiles using GC-TOF and SPALDI mass spectrometry. Prepared methyl esters were analyzed using GC-TOF mass spectrometer and the peaks were identified using known standards and retention times. Using this we were able to get different fatty acid profiles for the two different tick nymphs fed on different animal blood. This indicates that the tick nymphs retain their host lipid fractions without digesting them. This theory needs to be confirmed with more experiments. Along with this experiment we were able to analyze the fatty acid profile from a single tick using SPALDI mass

spectrometry. Optimization of this method this will lead to a quick method of identifying host characteristics from vectors like ticks.





http://www.ento.psu.edu/Lyme/lifecycle.htm

Lone star tick *Amblyoma americanum*