



H161 The Impact of Insect Exclusion on Eukaryotic Community Succession on Porcine Remains

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Learning Overview: After attending this presentation, attendees will better understand the eukaryotic community associated with porcine cadavers and how the eukaryotic community changes with time in the presence and absence of insects.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by improving Postmortem Interval (PMI) estimation methods based on these succession patterns.

PMI estimation is a vital part of forensic investigations, yet determination methods for long-term PMI are not reliable. Currently, microbial signatures are being investigated, specifically concentrating on the bacterial or eukaryotic succession patterns as PMI predictors.¹ Insect accessibility to a carrion has long been established as a driving force of the decomposition process.^{2,3} Understanding which microbiota are released into these systems, as well as their origins, can aid forensic scientists in establishing robust and reliable PMI estimation methods. While forensic entomology has been useful for long-term PMI estimation, this method does not reflect situations in which remains are not immediately accessible to insects.^{4,5}

This study characterized the eukaryotic community associated with porcine remains that were either exposed ($n=6$) or excluded ($n=6$) from insect access, using 18S recombinant DNA (rDNA) MiSeq[®] sequencing. Skin swabs were collected from each cadaver from the lateral thoracic or lateral abdominal regions starting immediately after euthanization, daily for the first 5 days, and weekly after this up to day 61 (1,703 Accumulated Degree Days [ADD]). DNA was extracted using the organic Cetyl Trimethyl Ammonium Bromide (CTAB) extraction method followed by dual-index 18S rDNA MiSeq[®] sequencing on MiSeq[®] FGx platform using MiSeq[®] reagent kit v2 (500 cycles).^{5,6} Sequences were analyzed using mothur version 1.39.5 and using SILVA (release 132) as reference data for eukaryotic classification.⁷ Statistical analysis was performed using R version 3.5.0.⁸

An Analysis of Molecular Variance (AMOVA) suggested that there is a significant difference between the insect access and insect exclusion groups. However, the major phyla (>1%) differed only in their relative abundance. At the family level, similar patterns in top taxa can be seen. In both the access and exclusion groups, there is an increase in relative abundance of Rhabditida around 209 ADD/7 Days and an increase in the abundance of Dipodascaceae immediately after 448 ADD/15 Days. During the later stages of decomposition, the exclusion group showed much higher relative abundances of Filobasidiales and Trichosporonaceae, whereas the access group showed much higher relative abundances of Diptera, Coleoptera (as expected), and Ascomycota.

In conclusion, this study provides information on eukaryotes associated with porcine remains under both insect access and exclusion conditions. Here, succession patterns in both groups can be useful as predictors of the PMI. Utilizing this information in combination with prediction modeling may aid the forensic science community in filling gaps of extended PMI estimation methods, decreasing the overall rate of erroneous results.

Reference(s):

1. Forger L.V., Woolf M.S., Simmons T.L., Swall J.L., Singh B. A eukaryotic community succession based method for postmortem interval (PMI) estimation of decomposing porcine remains. *Forensic Sci Int.* 2019;302:109838. doi:10.1016/j.forsciint.2019.05.054.
2. Graham J.P., Price L.B., Evans S.L., Graczyk T.K., Silbergeld E.K. Antibiotic resistant enterococci and staphylococci isolated from flies collected near confined poultry feeding operations. *Sci Total Environ.* 2009;407(8):2701-2710. doi:10.1016/j.scitotenv.2008.11.056.
3. Pechal J.L., Crippen T.L., Benbow M.E., Tarone A.M., Dowd S., Tomberlin J.K. The potential use of bacterial community succession in forensics as described by high throughput metagenomic sequencing. *Int J Legal Med.* 2014;128(1):193-205. doi:10.1007/s00414-013-0872-1.
4. Pechal J.L., Benbow M.E., Crippen T.L., Tarone A.M., Tomberlin J.K. Delayed insect access alters carrion decomposition and necrophagous insect community assembly. *Ecosphere.* 2014;5(4):art45. doi:10.1890/ES14-00022.1.
5. Zheng L., Crippen T.L., Singh B., et al. A Survey of Bacterial Diversity From Successive Life Stages of Black Soldier Fly (Diptera: Stratiomyidae) by Using 16S rDNA Pyrosequencing. *J Med Entomol.* 2013;50(3):647-658. doi:10.1603/ME12199.
6. Kozich J.J., Westcott S.L., Baxter N.T., Highlander S.K., Schloss P.D. Development of a Dual-Index Sequencing Strategy and Curation Pipeline for Analyzing Amplicon Sequence Data on the MiSeq Illumina Sequencing Platform. 2013. doi:10.1128/AEM.01043-13.
7. Schloss P.D., Westcott S.L., Ryabin T., et al. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl Environ Microbiol.* 2009;75(23):7537-7541. doi:10.1128/AEM.01541-09.
8. R Core Team. *R: A Language and Environment for Statistical Computing.* 2013. <http://www.gnu.org/copyleft/gpl.html>.

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