Effect of Insect Feeding on Hard Tissue During Late-Stage Decomposition

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Abstract:

The study of how insect colonization affects the latter most stages of decomposition is vital to the progress of forensic studies as well as the applications of anthropology and entomology in a forensic context. In an effort to try and make progress in linking anthropology and entomology in an applied forensics context, the experiment was performed and consisted of observing and analyzing decomposing hard tissue for potential physical evidence of insect feeding. Three trials consisting of three samples of mostly stripped beef rib were allowed to decompose for three weeks each. The ribs were not completely stripped in an effort to incentivize the insects in the surrounding environment to feed on the samples. Afterward, the samples were cleaned and stripped by breaking down the collagen in the connective tissues of the remaining meat through boiling the samples, and then were analyzed under a dissection microscope. During analysis, each sample was individually compared to a control of the same type, and it was discovered that over 50% of the entire sample population of bones were found to have microscopic indentations that could not be accredited to environmental damage, production inconsistencies, or experimental error. The origins of the damage could not be attributed to any known variable in the experiment, but since there was proof of insect feeding on the samples the experiment proceeded under the assumption that the insects observed feeding on the samples were the origins of the damage. The conclusion drawn from the experiment is that insect damage from feeding can be imprinted on hard tissue for analysis during late stages of decomposition when hard tissue is more prevalent than soft tissue as a food source.

Key words: decomposition, entomology, anthropology, damage

Entomology and anthropology both play cru-cial roles in the field of forensics and any applica-tion therein. Insects play a crucial role in the decomposition process for most of the species on Earth including but not limited to humans. Without the feeding of insects during decomposition to aid bacteria and scavengers in the breakdown of the decaying material the buildup of material would begin to pose a serious threat to the environment, making humans virtually unable to live because any waste produced would be unable to be reduced fast enough to prevent build up (Bachm-ann and Simmons 2010). Moreover, insects also play a significant role in the field of forensics wherein an understanding of insect colonization, behaviors, and impacts on decomposition can provide information to facilitate leads for investigations and collect valuable data on insect's role on the

ecosystem relative to humans (Byrd and Castner 2001). Being able to understand and utilize the information provided by insect colonization during decomposition can be crucial when establishing a timeline for a decedent and expert insect identification can provide helpful information about a decedent or suspect's actions prior to death. Furthermore, anthropology applied in a forensics context can also tell experts and investigators just as much information. The ability to draw conclusions about a decedent's life and death based on a body that had been stripped of most if not all soft tissue is invaluable-especially when there is little physical evidence left from before death due to the degradation of time or natural exposure. Anthropological evidence can be invaluable to investigators especially during investigations of crimes that had taken place a long time prior to investigation, "cold cases", or cases with very little physical evidence due to exposure to the elements (Libal 2006). Taking into consideration how beneficial both applications of entomology and anthropology are to the field of forensics it would be pertinent to endeavor to find a link between the two fields (Kaiser and Katterwe 2001). The purpose of the experiment was to observe decomposition and the following insect colonization of samples that were majority hard tissue to visualize any possible connections between the feeding of insects and potential visible, verifiable, and replicable evidence of such feeding the bones being experimented on at the time. The implications of the success of this experiment, having found proof of insect feeding affecting hard tissue, would be that experts would be able to prove insect colonization without assuming prior feeding due to decomposition as well as the potential identification of the insects colonized based of specific feeding patterns if present.

Materials and Methods

The experiment was conducted in the back-yard of a home in Bryan, Texas. In this location three sets of three mostly stripped beef ribs made up three trials and were placed with a protective covering and allowed to decompose for approximately three weeks per trial to facilitate the attraction and feeding of insects on the samples. The trials were performed consecutively with each trial set being left to decompose for approximately three weeks on the soil surface while being covered by dense plastic tent structures (International Barrier Technology Inc., Watkins, MN). The plastic coverings were then staked into the ground to prevent the local wildlife from potentially interfering. There was some concern over how covering the samples would affect the experiment- the concerns being the protective measures were too re-strictive to allow insect access- which would affect decomposition rates (Pechal, Benbow, Crippen, Tarone, and Tomberlin 2014) of the sample as well as possibly prevent the feeding of insects. The workaround developed in response to the problem was to perforate the top of the coverings with small wholes approximately 3cm in length to allow insect access to the samples and the experiment continued. Over the span of the experiment the location of the samples, the length of time spent in exposure, and the preparation of the samples both initially and in the analysis was kept the same. After the exposure of the sample had completed its duration, the insects found feeding on the sample, if present, were recorded via photograph with an iPhone (Apple, Cupertino, CA) and the sample was prepped for analysis of the hard tissue. The samples initially still had raw meat attached to the bone to attract insects to feed, so for the bone to be properly analyzed the extraneous

materials had to be removed so a full view of the bone could be visualized under a microscope. First, the samples were collected and placed into designated non-airtight commercial-grade sandwich bags labeled with the trial and sample number to prevent contamination and to make transport easier. The samples were then prepared by placing the whole of the bone into boiling water. Boiling the bones was the chosen method for preparing the samples because the process of boiling breaks down the collagen in the connective tissues which makes removing the meat much more efficient (Ruantrakool and Chen 1986). Boiling the bones also ensured that most if not all bacteria acquired from decomposition were killed (Avens, Albright, Morton, Prewitt, Kendall, and Sofos 2002), along with any lingering insects. Once boiled for approximately ninety minutes the bones were removed and the meat was carefully hand peeled away from the bone and wiped down to remove any lingering debris. After cleaning the samples were once again sorted into commercial-grade non-airtight sandwich bags and kept within a commercial grade freezer as a means of preservation. Samples were then taken under a dissection microscope to be analyzed and compared to a control for any possible markings that would indicate insect feeding on hard tissue (Ross, Radisch, and Cunha 2019). Photographs were taken of samples with distinct markings to be used as a means of record when presenting data. A Chi-square test for independence was run on the binary distribution of data of each sample having been assigned as either demonstrating damage or not. The sample size for the experiment was nine, but for the sake of the test, each sample is independent with an assigned binary variable or either "1" for marks being present, or "0" for marks not being present. The lack of a significant relationship between the samples was assigned as the null hypothesis, and the presence of a significant relationship was assigned as the alternative hypothesis with the critical value being 0.05 or 5%.

Results

The results of the experiment indicated the implication of possible insect markings on hard tissue samples. Given the three trials with three samples each, it was discovered five of the nine total samples had damage to the surface of the bone that was inconsistent with normal patterning, knife marks, chipping damage, and other larger surface damage as seen when compared to a control sample. The unknown origins of this surface damage coupled with the known presence of insects and insect feeding throughout the experiment offers a possibility of some unexplained surface damage being attributed to insect feeding. The unknown damage to the surface of the bones in question seemed to resemble mi-croscopic pockmarks or shallow divots. The samples that had the marks were samples 1 C, 3A, 2A, 2B, and 2C, each with damage patterns similar to each other but not seen on the control sample. The chi-square test for independence with a critical value of p= 0.05 and 8 degrees of freedom produced a P-value of 0.34. The P-value is greater than the critical value, making the calculated P-value significant and allowing us to reject the null hypothesis and assume there is a significant relationship present between samples (Preacher 2001).

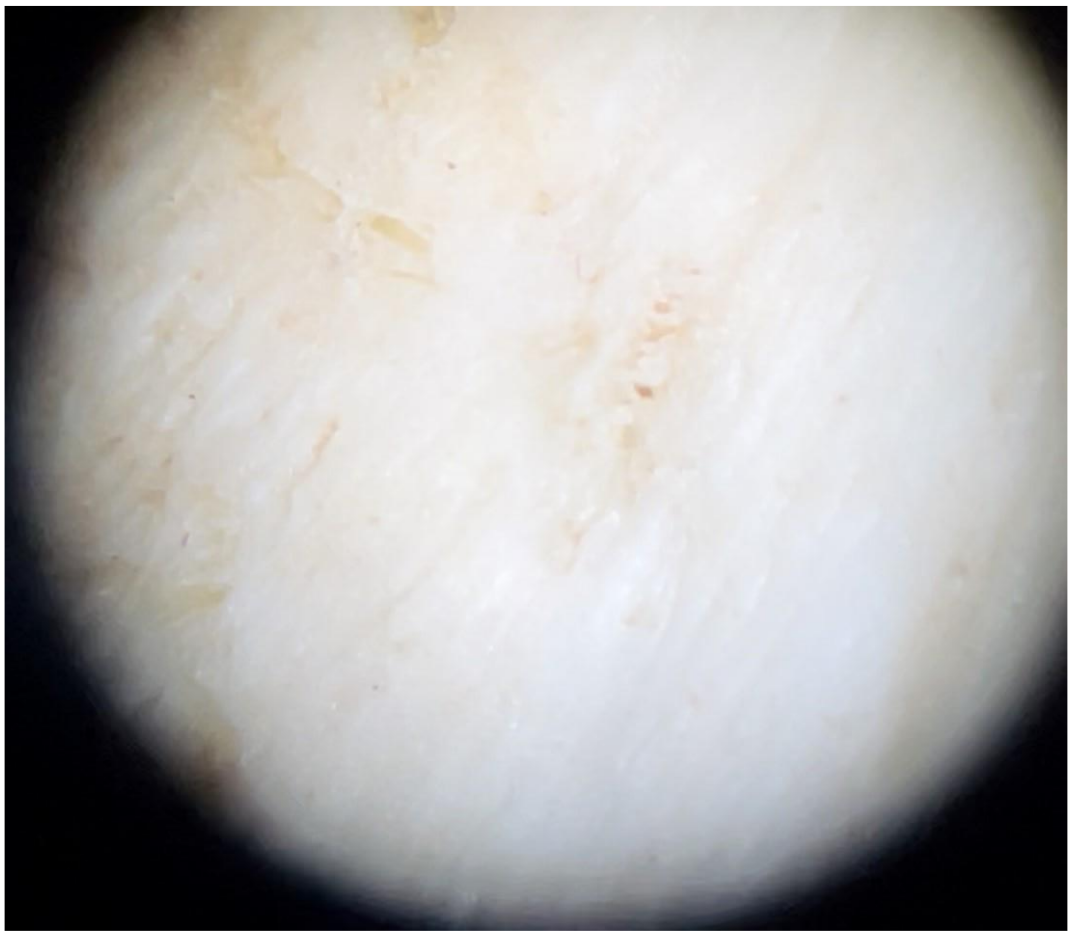

Figure 1, Photograph of sample control used as reference in experiment

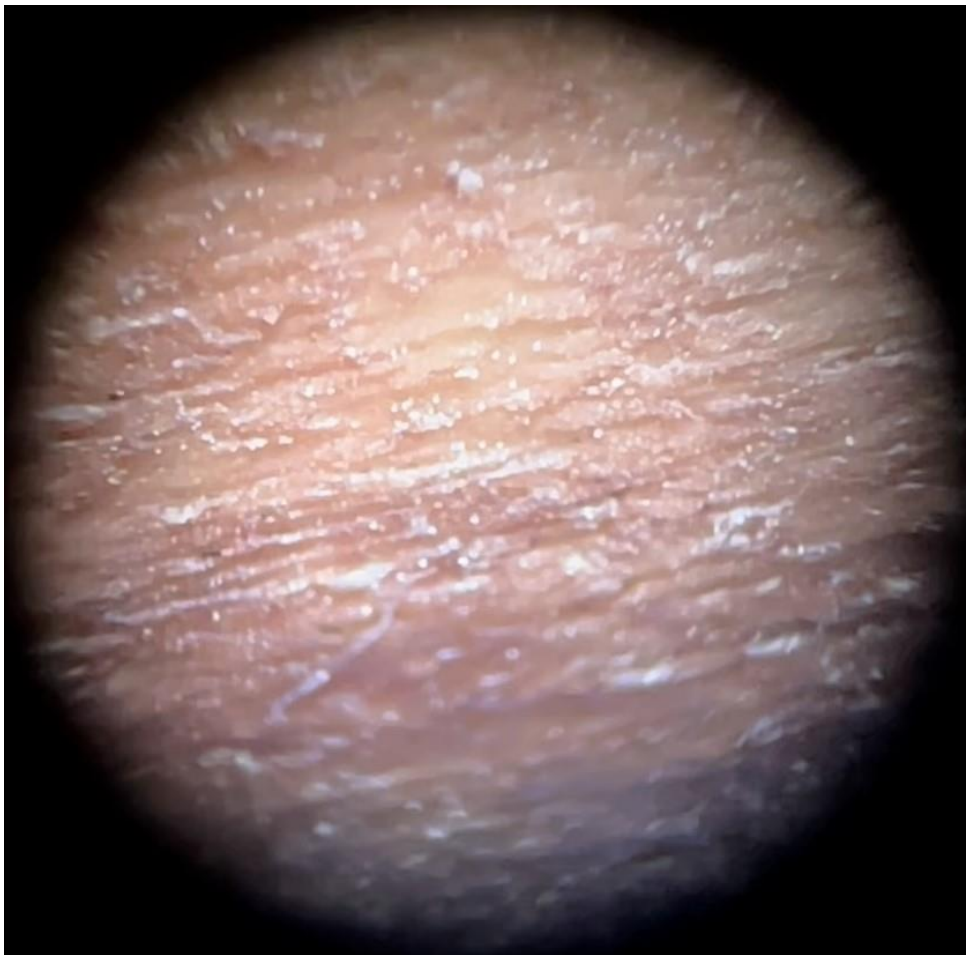

Figure 3, Photograph of knife striations caused during preparation of sample

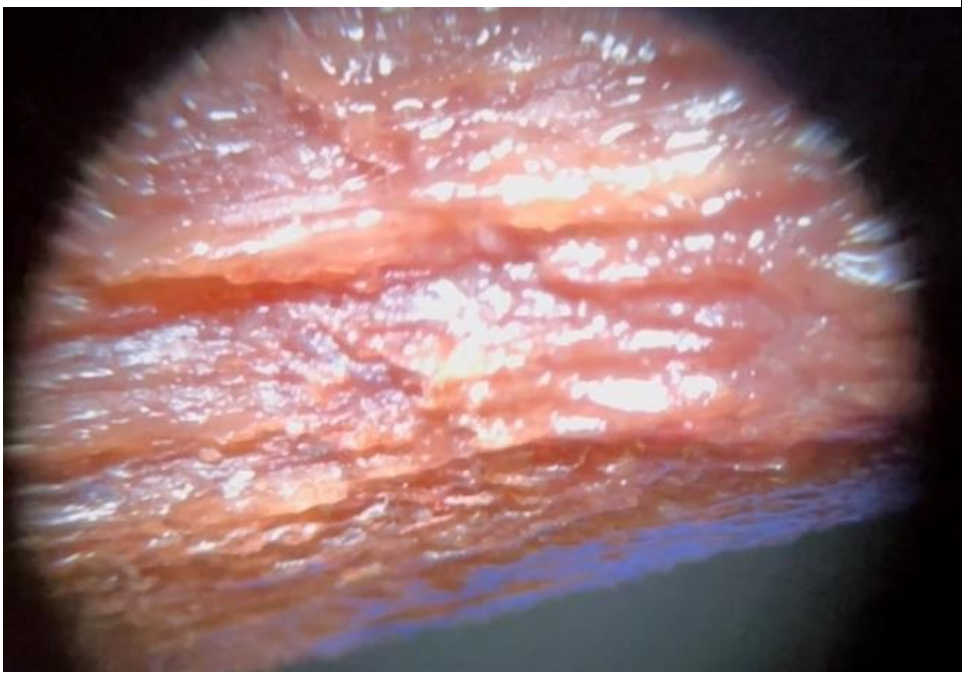

Figure 4, Photograph of environmental damage done to sample

Figure 5, Photograph of sample 1C with highlighted areas of interest

Figure 6, Photograph of sample 2A with highlighted areas of interest

Figure 7, Photograph of sample 2B with highlighted areas of interest

Figure 8, Photograph of sample 2C with highlighted areas of interest

Figure 9, Photograph of sample 3A with highlighted areas of interest

Discussion

The results of the experiment were largely inconclusive. Because of the inability to conclusively prove that insects were the cause of the specific damage done to the bones, there is no way to completely prove or disprove the origin of the marking in any definable way, and the experiment would need to be improved upon to provide any verifiable and conclusive data. One reason why the data may not be representative of a greater conclusion is because of the small sample size (Button, Ioannidis, Mokrysz, Nosek, Flint, Robinson, and Munafò 2013) as well as the number of uncontrolled variables present in the experiment in the form of weather conditions, wildlife interference, human interference, quality of meat, and the inconsistency of the type of insect present due to seasonal changes. While a more controlled setting, like a laboratory or climate-controlled environment with an insect presence, was not available for the experiment to take place though it is highly likely that such an environment would prove beneficial to the validity of the experiment as well as the quality of the results produced. A requirement of the experiment is that insects feed on the samples, and while there is proof of insect feeding during the experiment, there is still the issue that no insect activity was observed in real-time- only in the aftermath of sample collection. The ability to observe insect activity and feeding in real-time would not only provide further evidence to the proof of insect feeding but would also allow observation into what material present in the samples the insect would specifically be feeding upon. Knowing the specific material being fed on and having observable proof would be highly significant when interpreting the results of possible future experiments. Looking forward, the data that could be collected from future improved experiments would provide a more in-depth understanding of how the environment impacts a body after death as well as the behavior of insects during the late stages of decomposition and how anthropology can be applied to a greater subset of specialties connected through forensics.

In summation, the further study of how insect feeding affects hard tissue present during decomposition would prove invaluable to further connecting the fields of anthropology and entomology, especially in a forensics context. While no definitive data was collected to support the idea of insect feeding leaving identifiable damage or impressions on hard tissue, further study with modifications to the experimental process could prove fruitful.

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