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How Long After a Carrion Beetle Feeds on a Beef Carcass Can You Recover Identifiable Beef Specific DNA?

Trenton Smith

ABSTRACT

Carrion beetles are a main species that help in the decay of dead organisms and are a key species used by forensic entomologist to determine how long a body has possibly been deceased. We believe these beetles may offer even further insight into what happen on a crime scene based off their diet. Their diet can be used for DNA recovery of a specific species creating another DNA recovery method for crime labs. For this study we set up 5 different treatments with 5 samples for 4 of the treatments (n=50/treatment, 10 beetles/sample) with one treatment being just a meat sample. Beetles were given beef to feed on for 3 days and then the beef was removed. The 4 treatments after 1, 24, 48, and 72 hours away from the beef were each then separated into 5 samples. We chose these time periods to see how DNA may degrade over time and not be recoverable because of digestion. Extraction and purification of DNA from each sample was then started by Qiagen DNeasy purification kit. Once extracted and purified the DNA samples then went through PCR amplification and gel electrophoresis using Desert Hedgehog forward and reverse primers. Desert hedgehog is a mammalian specific primer used for many vertebrate and invertebrate studies. Major findings of the research conclude that 16 of the 21 samples ran through PCR were amplified showing that there is no degradation of beef DNA within 3 days of being removed from the scene. This result makes beetles a reliable source for DNA recovery.

Keywords: *Desert Hedgehog primer, PCR amplification, Gel electrophoresis.*

INTRODUCTION

Forensic science is needed to help solve crimes by using all and any information (evidence) that can be found on a crime scene to help determine who if anyone has committed a crime. (Eclaiocht, 2008). Evidence that will be given to forensic scientist may include samples of blood, hair, fibers, glass and fingerprints (Cox, 2017).

Many of the samples that are given to a forensic scientist will be evaluated and examined for many different things. One of the more useful types of evidence is DNA. Scientist must be able to analyze this DNA material "As it has evolved to become an indispensable and routine part of modern forensic casework, employing extremely sensitive PCR-based techniques to analyze biological material." (Jobling, 2004) This DNA evidence that is given to the scientist is extremely important to finding the specific suspect that the DNA came from.

Recovery of the physical evidence from the environment on a crime scene is one of the tougher things to do for crime scene investigators. There are specific techniques used to help properly collect the physical evidence, such as securing the crime scene from all outside threats that could potentially harm the evidence. (Kally, 2017) Any of these outside threats can effect how evidence is potentially perceived. Outside threats include weather, humans, and or animals. All of these outside threat can smear blood stains and or move evidence from the scene.

In a scene that involves deceased carcasses it is evident almost right from the early stages that animals from all different kind of families are attracted to the smell of the decomposing carcass. These animals will find the carcass and then help in the decomposition of the carcass even further. Insects play a crucial role in the decomposition of these carcasses and sometimes forensic entomologist will be used to study these insects in a criminal investigation. Forensic entomologist will study the populations of insect and their larval stages to estimate postmortem index and the cause of death.

Forensic entomology can be extremely helpful in the solving of a crime. One of the main insects that entomologist identify on scene to help solve a case are carrion beetles.

These beetles may offer even further insight into what happened on a crime scene based off their diet. It has already been determined that evaluation of decaying body can help determine the time scale of how long a body has been dead. In a study done by K. Tullies and M. L Goff decomposition process of a body was done in five stages after death; fresh stage (1-2 days), Bloated stage (2-7 days), Decay stage (5-13 days), post decay stage (10-23 days), remains stage (days 18-90). During this time the beetles are trying to continue their rapid growth along with their larvae which means that the beetles will need efficient digestion methods. (Vogel, 2017.)

Continued research to develop another way for forensic scientist to recover DNA from a potential crime scene. This research has led us into our topic on whether DNA is recoverable from the gut contents

of our beetles and if there is degradation of the DNA over time. And not only is it recoverable but how long is the DNA recoverable for. Recoverable DNA is fully determined by the efficient digestion of nutrients to maximize nutrient assimilation.

MATERIALS AND METHODS

Carrion beetles (Silphidae Americana) were purchased by McPherson natural science department. Beetles were separated into 5 different treatments that differed in the amount of time they were away from the beef food source before attempts at DNA extraction (N=50/treatment): 1 hour, 24 hours, 48 hours, 72 hours. I also extracted DNA from the meat alone as a positive control and the beetle alone (no feeding on beef as a negative control to test the effectiveness of DNA primer.

Of the 50 carrion beetles in each treatment 45 of the carrion were adult species and 5 were larvae. A slab of beef meat was split into 4 different pieces and placed into each treatment container. A section of the meat was saved as a positive control confirming the process of PCR amplification of a mammalian gene.

Beetles fed on beef for 3 days after introduction to the meat. After three days of letting the beetles feed on the beef we took the meat out of each container. After 1 hour away from the meat we then separated the 50 beetles within each treatment into 5 different sample groups (5 samples/treatment). Each sample contained 9 adult carrion and 1 larvae. The sample groups were immediately placed into plastic bags and frozen to stop the breakdown of DNA. We then proceeded to crush and then wash the crushed beetles back into the plastic bags. This process was done for treatment 2, 3, and 4.

DNA extractions were done using DNeasy Blood and Tissue kit. Each sample (10 total beetles) was processed individually. I placed less than 25mg of carrion beetle tissue sample into 5 different 1.5ml microcentrifuge tubes for each treatment group. We then added 180 microliters Buffer ATL and added 20 microliters of proteinase K, mixing by vortexing and incubating until completely lysed, vortexing occasionally during incubation. (Qiagen Inc.) We then added 200 microliters of Buffer AL and mixed thoroughly by vortexing and then incubated for ten minutes. (Qiagen inc.) After 10 minute of incubation we added 200 microliters of 100% ethanol and mixed thoroughly by vortexing the samples. (Qiagen Inc.) Using a pipette, we then placed the mixtures of each sample into DNeasy mini spin columns which were placed in 2ml collection tubes. Once placed in tubes we then centrifuged the samples at 8000rpm for 1 minute. After centrifugation we discarded all flow through in collection tube, then placing all 5 spin columns into new collection tubes and added 500 microliters of buffer AW1 to each sample mixture. We then placed samples in the centrifuge at 8000rpm for 1 minute. Once done with that we discarded all flow through. Once again, we placed samples into new collection tubes and then added buffer AW2 mixture. This time we placed samples in the centrifuge at 14,000 rpm for 3 minutes. Once centrifugation was complete we placed the samples in new 1.5ml microcentrifuge tubes. We then eluted DNA by adding 200 microliters of buffer AE to the center of the spin column membrane and placed them in room temperature incubation. After 1 minute of incubation we placed the samples into the centrifuge for 1 min at 8000 rpm. For our project we decided to do this step twice to increase the DNA yield that we would later see. This process was done for every sample in each treatment.

The DNA in each sample (if any) was then run through a PCR to attempt to amplify the beef specific DNA. For amplification of the samples we diluted down both Desert Hedgehog (DHH) forward and reverse primers down to a concentration of 100x. Desert Hedgehog was used because of its ability to be amplify only mammalian DNA (O'Hara, 2011) After dilution of forward and reverse primers we placed 5 microliters of each into each Ready-to-Go PCR beads. In addition to the forward and reverse primers we added 10 microliters of our template and added 5 more microliters of sterile water. The PCR was set to 35 cycles of the following temperature steps: 90°C for 60 seconds, 65°C for 60 seconds, 72° for 60 seconds.

For visualization of the PCR products, I used gel electrophoresis. We made 2% agarose gels in TAE buffer. Gels were run in 3 separate electrophoresis units holding TAE buffer with 120 volts of electricity. Each sample was run along with 2 DNA markers, a indicator and Gels were stained with a mixture of 1.5% ethidium bromide in 100ml of TAE buffer . Gels were stained with ethidium bromide for 7 minutes. Stained gels were placed over a UV light source to visualize DNA bands.

RESULTS

Mammalian DNA was found in every treatment that was ran. 16 of the 21 samples were amplified during gel electrophoresis. Treatment one (1-hour, figure 1.) only had 1 sample that was amplified, the amplified sample had a very small fragment amplified and was represented by a very light band in lane 4 (sample 3). Treatment 2 (24 hours, figure 2.) had 4 out of 5 of the samples amplified, bands that were amplified were represented by lighter bands. Treatment 3 (48 hours, figure 3.) and treatment 4 (72 hours) each had all their samples amplified. Treatment 3 and 4 showed samples amplified by darker bands. Of the 21 samples 2 were an original beef sample and an individual carrion sample that had not been introduced to meat. The beef sample was amplified and was represented

Figure 1. Left to right, lane 1 shows a DNA ladder representing different fragment sizes. DNA samples from treatment 1, samples 1-5(lanes 2-6) were PCR amplified with the DHH primers. Sample 3 (lane 4) was the only sample that was amplified in treatment 1. DNA samples of treatment 2, samples 1-2 (lanes 7-8) were also PCR amplified. Sample 2 of treatment 2 was amplified in lane 8

Figure 2. Lane 1 shows a DNA ladder with different fragment sizes. DNA samples 3-5 of treatment 2 are in lanes 2-4 which were all amplified. Samples 1-5 of treatment 3 are in lanes 5-9. Treatment 3 sample 2 and 5 in lane 6 were represented by a dark band. Carrion individual insect DNA is in lane 10 and was not amplified. All samples in the treatment were amplified using DHH primers.

by slightly dark band and a longer smear (Unit 3, Lane 8). In lane 10 in unit 2 the insect sample was not amplified.

DISCUSSION

Analysis of the treatments shows us that there is no degradation of DNA overtime and it might suggest DNA may be recoverable from beetles over a short time period.

The size of the DNA fragments for pBr322 are 1,857bp, 1,058bp, 929bp, 383bp, and 121bp. We see all of our beef DNA samples that were amplified showing were larger than this, suggesting they were around 5000 base pairs. The statistical chance of something else being amplified other than the beef DNA is low. Chances of something else being amplified is low because the primers I used are specific to a certain sequence only found in mammal DNA. The lack of an amplified band in the insect to the positive band in just our beef and bands in our samples allow us to make these conclusions. Along with this the molecular markers that were amplified show that they are all the same size telling us the DNA amplified is all related.

Figure 3. Left to right, lane 1 shows a DNA ladder representing different fragment sizes. Samples 1-5 of treatment 2 are represented in lanes 2-6, which were all amplified. The control meat sample is in lane 7 and represented with a longer smear and a darker band. All samples were amplified using DHH primers.

The results of exon 3 being amplified also suggest that Desert Hedgehog is indeed a key regulator of pattern formation in many vertebrate and invertebrate species (O'hara, 2011). Desert hedgehog belongs to the hedgehog gene family and has been found to function in both mice and humans (O'hara, 2011) suggesting that cows are evolutionarily related.

This research is important to the field of forensic entomology because it is another useful way to help identify or confirm a suspect in a criminal case.

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