How insects and microbes reveal crime scene clues

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In a thick wooded area, a dead body lies. Within 5 to 10 minutes, a slew of tiny winged visitors –blow flies – are the first on the scene.

Experts say that where there be carrion, this metallic blue-green looking fly is often the first insect to arrive because it can smell death from up to 10 miles away.

As minutes turn to hours, and turn to days, weeks and months, the insect population will change with the passage of time. Flies, beetles and mites will come and go. Exactly which insects infest a decaying body is dependent on the conditions surrounding it, including the temperature and climate. Some insects will come to feed on the decomposing corpse, and others will come to lay eggs/larvae.

When a suspicious death occurs, insect activity on a corpse can provide valuable information to crime scene investigators about the post mortem interval. PMI is the estimated time that has elapsed since a person has died.

Forensic experts will collect insect samples from the crime scene to take back to the lab for further examination. They can compare their findings with databases detailing insect populations at different stages of development and under different temperatures to help determine PMI.

The more species that are present the better, because the overlapping data provides researchers with statistical confidence and precision in the estimation of time of death.

But this can be an arduous task, and by the time some bodies are taken to a medical examiner's office, they may have reached late stages of decomposition. Additionally, not every crime lab is equipped with the necessary technology to process the evidence.

So forensic experts like Baneshwar Singh, Ph.D., a forensic entomologist and assistant professor of forensic science in the Virginia Commonwealth University College of Humanities and Sciences, are working toward the development of new tools for the estimation of PMI.

Singh's research spans the gamut – from understanding the role of insects and microbes in estimating PMI to identifying the chemical

composition of odor being emitted from a decomposing body.

"Eight to nine years ago, it was thought that microbes could help us determine PMI – but we didn't have the technology," said Singh, who has trained FBI agents on how to collect insect evidence and is working toward becoming a board-certified forensic entomologist so he can lend his expertise and testify in a court of law.

"But, in the past four to five years alone, there have been significant advances in the technology of next generation sequencing and genome sequencing that has made it possible to quantify bacteria from a sample within a day," he said.

Bacteria and PMI

In 2011, prior to joining to VCU, Singh was part of a multi-university team at Texas A&M that examined and quantified bacteria found on human cadavers at different time points. The work, funded by the National Institute of Justice, found that bacteria present on the decomposing body changed with time. Singh said that some bacteria increased in quantity, while others decreased in quantity. The process, known as bacterial succession, may eventually be used to help estimate how long a body has been in a state of decomposition with better precision than before.

The team, which started examining a bacterial pool of thousands, has been able to pinpoint five or six bacteria which may be valid indicators of PMI.

Singh has continued this work at VCU.

"Ultimately, our goal is to develop a real-time PCR[SO1] [SO2] -based kit so that within two hours any crime lab or medical examiner's office can estimate how old the body has been in the state of decomposition," said Singh.

"PCR, which stands for polymerase chain reaction, is a biochemical technology/technique that helps researchers produce millions of copies of a DNA sequence in a brief period of time - maybe a few hours, whereas real-time PCR monitors DNA amplification in real time, and helps researcher in quantification of DNA," he explained.

The real-time PCR kit will include fluorescently labeled primers (for

specific targeted region of selected PMI indicator bacteria), and PCR reagents. A real-time PCR kit could make processing the data in a crime lab faster and more efficient.

Additionally, it could be a cost-effective alternative to aid crime labs that are not equipped with expensive DNA sequencers and related technology.

"This field requires a lot of basic research before we can take this and use it in the courtroom. We want to have statistical confidence – we want 95 percent confidence – that the PMI we determine is as accurate as possible. To do that, we have to do a lot of research on ecology and evolution of insects, microbes, and understand how they interact with each other," he said.

Understanding the various types of bacteria found on the decomposing body may also provide information about the race or ethnicity of the deceased. In another project, Singh is delving into how bacteria found on the human body may differ from race to race or among individuals from different ethnic backgrounds. Still in its early stages, Singh hopes any findings derived from the work can be used to help identify the race or ethnicity of the deceased.

"The goal with this work is to develop a universal method that will help investigators effectively make the identification," said Singh.

Inter-kingdom attraction

Recently, Singh and his colleagues have been studying the relationship between bacteria found on the decomposing human body and the black soldier fly. They have observed that the bacteria of a decomposing corpse – via some chemical compound it produces during decomposition - influences the location of where the flies lay their eggs. Singh refers to

this as 'inter-kingdom attraction or signaling.'

"There is some chemical being produced by the bacteria that helps in their own activity of decomposition – helps attract the insects," said Singh.

In the study, published in *Nature Scientific Report* last year, the team examined the black soldier fly and observed that there was a group of bacteria – not just one bacterium – that attracted the fly to a particular area to lay eggs.

"The black soldier fly went to where there was a mix of bacteria. If that bacteria is not there, the flies do not come," he said.

"This is important information - there is some chemical that attracts the insect. And there are some chemicals that come out of the microbes that repel the insect. Understanding this will help with better management of the insect," explained Singh.

Odor and microbes

In other work, Singh and colleagues are examining the chemical composition of odors produced by microbes during human decomposition.

Several years ago, a colleague of Singh's was involved with the high-profile Casey Anthony case where there was garbage found in the back of a vehicle linked to a suspicious death – but no body. The crime scene investigators could not distinguish if the odor detected was due to the decomposition of a human body, or the fact that there was a ton of trash locked in the small space.

"If we can collect the odor, if we know that this chemical comes from human decomposition, then we can determine: 'Yeah, there was a human body here – somebody moved it,'" Singh said.

But, this is tricky – and there are many variables involved, said Singh.

"We don't know the bacteria growth rate, yet. Some grow at lower temperatures, some grow at higher temperatures," he said.

While there remains a lot of work ahead, Singh is excited for the possibilities.

"Right now we've just opened the box," he said. "There are a lot of questions to be answered. As more people come together the more we'll be able to advance what we know."

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