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RESEARCH NEEDS FOR FORENSIC ENTOMOLOGY IN ECUADOR

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ABSTRACT

Research needs to establish forensic entomology as an important technique to add in crime scene investigation in Ecuador are presented. Because Ecuador is a tropical megabiodiverse country with significant variation in habitat throughout the country, the first step is an inventory of insects with potential importance in medicolegal investigations. These investigations must be conducted in a variety of habitats, sub-habitats and times of year. The cooperation of the police to determine sites where cadavers are frequently found will be essential in setting priorities for these studies. This should be followed with experimental studies using pig carcasses in controlled field situations to document the continuum of insect infestation of cadavers. This can be supplemented detailed observation of actual crime scenes with the cooperation of the police and prosecutors. These studies will need to be supported with controlled laboratory experiments with the principal insect species identified during the inventory to determine growth rates under defined temperature conditions. These experiments are crucial to develop a database to determine postmortem interval in the investigations of crime scenes. After this basic data has been obtained, it will be possible to refine these studies with laboratory and field experiments that investigate the effect of different wound types, presence or absence of clothing, location of cadavers inside structures or outside and the impact of recreational drugs on fly larvae development. The development of forensic entomology in Ecuador should be viewed as a continuing collaboration between scientists, crime scene investigators, police, prosecutors, the judiciary and the legal community. Forensic entomology has the potential to significantly assist the investigation of crimes and prosecution of criminals in Ecuador.

KEYWORDS: Calliphoridae, Ecuador, forensic entomology, Sacrophagidae.

RESUMEN

Se presentan las necesidades de investigación en el Ecuador para establecer a la entomología forense como una importante técnica que ayuda a la investigación de la escena del crimen. Debido a que el Ecuador es un país megadiverso con una variación importante en su hábitat a lo largo del país, el primer paso es un inventario de insectos con importancia potencial en investigaciones médico-legales. Estas investigaciones deben ser conducidas en una variedad de hábitats, subhábitats y épocas del año. La cooperación de la Policía para determinar los sitios donde los cadáveres se encuentran frecuentemente será fundamental a la hora de poner prioridades para estos estudios. A esto deben seguir estudios experimentales mediante el uso de cuerpos de chanchos en situaciones de campo controladas para documentar el continuo de la infestación de los cadáveres por insectos. Esto puede ser suplementado por observaciones detalladas de escenas reales del crimen con la cooperación de la Policía y los Fiscales. Estos estudios necesitarán ser apoyados con experimentos controlados de laboratorio con los insectos más importantes encontrados en los inventarios de campo, para determinar tasas de crecimientos bajo condiciones estándares de temperatura. Estos experimentos son cruciales para desarrollar una base de datos para determinar el intervalo postmortem en las investigaciones de las escenas del crimen. Después que estos datos han sido obtenidos, entonces será posible refinar estos estudios con experimentos de laboratorio y de campo que investiguen el efecto de las diferentes heridas, la ausencia y presencia de ropa, la ubicación de los cadáveres dentro de estructuras o fuera en áreas de recreación y el impacto de drogas recreacionales en el desarrollo de las larvas. El desarrollo de la entomología forense en el Ecuador ha de ser visto como una continua colaboración entre científicos, investigadores de las escenas de crimen, fiscales, y la comunidad judicial y legal. La entomología forense tiene el potencial de asistir significativamente a las investigaciones de los crímenes y al enjuiciamiento de los criminales en el Ecuador.

PALABRAS CLAVES: Calliphoridae, Ecuador, entomología forense, Sacrophagidae.

INTRODUCTION

Forensic entomology is the broad area where entomology and the legal system interact. In general, forensic entomology can be divided into three areas (Hall and Huntington, 2010). Urban forensic entomology is concerned with insects infesting human structures, nuisance insects, patients in health care facilities and abuse of pets and domestic animals. Stored products forensic entomology involves contamination of food and food products by insects and other invertebrates. Usually this involves packaged food with evidence of insect infestation but can also involve freshly prepared restaurant food that has been contaminated. The third area of forensic entomology can be termed medicolegal entomology and is concerned with the use of entomological evidence in solving violent crimes, frequently murder. This area has received substantial attention in the scientific and legal literature and will be the focus of this review. Because the costs of such crimes and their prosecution are so high in terms of human life, the evidentiary standards are quite high. Entomological evidence in these cases must be supported by research into the insect fauna associated with cadavers and details of the life cycles of these insects to have the maximum utility legally.

A major factor in using entomological evidence in medicolegal cases is reliability of the information used in the testimony of entomologists and other forensic scientists. Reliability is built on a solid background of research into the taxonomic identity of the species involved and the details of their life history. A significant problem concerning this aspect of forensic entomology is that Ecuador is a megabiodiverse country and probably has a great diversity of insects that are attracted to cadavers at different stages of decomposition in differing environments and geographic area. Further, there has been very little work done in the area of medicolegal entomology in the past so little is known about these insects of potential legal value. Reliability also demands that investigators that collect insect evidence and those that interpret this evidence and report it to legal authorities be very well trained so that their testimony is unassailable legally. As we do not have a history of research in this area in Ecuador, we must start from the beginning. We must gain the trust of the Ecuadorian legal community so that entomological evidence can be given the weight it deserves and be used as another important tool in solving major crimes.

For these reasons, it is essential to develop a core group of researchers and forensic experts in the area of forensic entomology. It is expected that these individuals will seek advanced training outside of Ecuador with established experts in the area of forensic entomology and regularly attend scientific and legal meetings where research results are presented and topics related to forensic entomology are discussed. Continuing education will be important in demonstrating expertise and professionalism in this area. Forensic entomology professionals must practice their profession in collaboration with university scientists, police, medical examiners and prosecutors. They must be ready to respond to crime scenes on short notice in a thorough, professional manner. Importantly, research must be undertaken to establish the baseline information necessary to use entomological evidence in legal proceedings. This will include developing a worldwide network of experts that can rapidly and accurately identify insects that are collected as part of medicolegal investigations.

DEVELOPING FORENSIC ENTOMOLOGY IN ECUADOR

Laboratory Facilities

One of the first goals will be to develop dedicated laboratory facilities for research and obtaining data for specific cases involving forensic entomology. These laboratories will need facilities for rearing insects under conditions of constant temperature and humidity and variable photoperiod. A series of the constant-temperature chambers will be needed for simultaneous studies. These will be essential in studies of insect development related to estimating the post mortem interval. Many forensically important species are collected as immature larvae that will need to be reared to the adult stage to be accurately identified. For many species of flies, only the females are attracted to cadavers however, the identification of female flies is difficult and in many cases impossible. In these cases, the female flies must be provided with an ovipositional substrate (eg. liver) and the eggs reared to the adult stage in hopes that a male fly will emerge from her offspring. These laboratories will need to be equipped with microscopes for use in these studies and photographic equipment to document the results of these investigations.

These laboratories must develop a set of equipment for field investigation. These kits must be maintained in good condition, ready for immediate use at a moment's notice when a case presents

itself. These kits must contain equipment to maintain the integrity of the crime scene, head nets, latex gloves, protective shoe covers and coveralls. Equipment for collecting environmental data, eg. thermometers and light meters, is essential as well as equipment for collecting insect and soil samples. Special attention must be given to have enough vials to make complete collections of samples as under some circumstances the number and diversity of insects will be huge. Photographic equipment will be necessary to document the process of data collection and the status of the crime scene. It will be necessary to cooperate with police and prosecutors to develop standard forms to document the collections of forensic entomology data at crime scenes.

An essential part of these laboratories will be a library for bibliographic material associated with forensic entomology. There is a growing amount of literature on this subject that will need to be collected and maintained for use by this core group of forensic scientists. Both classic and current works concerning the taxonomy of forensically important species will also need to be archived in this library. The majority of these resources are in English but there is a growing literature in Spanish as well. Increasingly, these bibliographic resources are available on-line, some from open access sources and some by subscription only. Collaborations with universities may be important to gain access to these materials. Many of the basic reference works on the taxonomy of forensically important insects are available only as physical books.

DATA NECESSARY TO USE ENTOMOLOGICAL EVIDENCE

Inventory of Forensically Important Insects

One of the first goals in developing forensic entomology in Ecuador will be to produce an inventory of insects with importance in potential homicide investigations. The basic procedure for this inventory is standardized through other studies around the world (Gennard, 2012; Byrd and Castner, 2010). Typically pig carcasses are used in these studies as they closely approximate human cadavers in size and composition. The pig carcass is placed inside a protective cage that allows insects to enter and colonize the carcass while preventing scavenging by vertebrates. This scavenging can significantly alter the physical location and status of the carcass and therefore could impact the colonization process by insects. The investigators must regularly visit these carcasses to observe the

behavior and sequence of the colonizing insects, collect these insects in all developmental stages for identification and document the process and sequence of decomposition of the pig carcasses. Because Ecuador has a great geographic diversity, these studies must be conducted in the major biogeographic regions of the country: dry coast, humid coast, cloud forests on the eastern and western slopes of the Andes, the sierra, and Amazonia. These geographic studies should be centered around major cities as this is where homicides are most likely to happen. It will be important to coordinate these studies with local and national police to concentrate on areas where victims of homicide have been found in the past. Certain areas with high crime rates and secluded locations for disposal of bodies should be given priority in these studies. In this way, forensic entomology data can be collected that will be immediately useful for law enforcement.

In addition to planned studies using pig carcasses, the collaboration of crime scene investigators will be important. Forensic entomologists should be called to crime scenes when there is the likelihood of encountering entomological evidence for general collecting. With the permission of the crime scene investigators, the entomological team can collect valuable specimens to build the reference collection with insects collected from human cadavers.

Geographic data of this sort will be useful in determining if a cadaver has been moved after death. This is one of the major pieces of information that can be provided by medicolegal entomology and can be a key piece of evidence in investigation of crimes (Byrd and Castner, 2010). Understanding which insects are cosmopolitan in their distribution and which are specific for particular geographic area can be important in determining if a person was killed in one location and then subsequently moved for disposal and concealment. Establishing this sequence of movement and perhaps a timeline through entomological evidence can be an important component in establishing the details of a crime and the movements of the criminal in relation to the cadaver.

Field Studies

After this basic data is collected, it will be important to perform these baited collections in a diversity of habitats. For example, even in adjacent locations, the insect fauna attracted to a cadaver may be significantly different if the cadaver is in the sun as opposed to the shade (Byrd and Castner, 2010). Similarly, pristine

habitats may have very different forensic insects as compared with adjacent agricultural areas or urbanized areas. We are just beginning to understand seasonality in insect abundance in the tropics so changes in abundance of forensically important insects will be an important topic for investigation. Obviously, major effects such as the rainy season and dry season could potentially have a major impact on which species of insects are attracted to cadavers. However, it is likely that more subtle changes in seasonality may also provoke changes in forensic insect populations attracted to cadavers (Brundage *et al.*, 2011).

An area of emerging importance is forensic entomology in aquatic habitats, lakes, rivers, stream and swamps (Haskell *et al.*, 1989). Many criminals attempt to conceal the cadaver of their victims in water and forensic aquatic entomology has proven useful in obtaining important evidence in these cases (Byrd and Castner, 2010). Recently, Barrios and Wolff (2011) conducted a survey using pig carcasses in a stream and a lake at about 2600m in the Andes of Colombia. They collected over 18,000 insects associated with the pig carcasses, nearly 11,500 specimens representing 33 species in the lake and over 7,300 specimens representing 25 species in the stream. They identified six stages of decomposition with slightly different timing in the lake vs. the stream. Each of these stages had a characteristic insect fauna although there was considerable overlap for some species. Studies such as these indicate that there is considerable forensic information in aquatic insect collections associated with cadavers in fresh water. An important parameter is the postmortem submersion interval, how long after death had the cadaver been submerged. Blowfly (Calliphoridae) pupae have differential survival during submersion and this can be used to obtain relatively accurate measures of postmortem submergence interval (Reigada *et al.*, 2011). These investigators were also able to use the pupal parasites of these blowflies to refine their estimate.

Recently forensic investigators have turned their attention to biological examination of cadavers disposed of in marine environments (Byrd and Castner, 2010). Currently, there is no reliable method for determination of postmortem submersion interval in salt water. While the insect fauna in marine environments is much less diverse, some species have been associated with cadavers in certain circumstances such as bloodworms (Diptera; Chironomidae) (Goff, 2000). Crime scene investigators initially classified these larvae as fibers and began to process them as

non-biological samples. Typically, cadavers in salt water are infested with a variety of other marine invertebrates (Byrd and Castner, 2010) and little is known about the succession patterns of these invertebrates on cadavers in various circumstances. Dickson *et al.*, (2011) found that bacteria were a good indicator on postmortem submersion interval in a marine environment in New Zealand. They could identify five distinct stages of decomposition with characteristic bacterial communities. Decomposition rates and the associated bacterial communities did vary seasonally indicating that significant research would be necessary to make the use of bacterial community colonization and succession a reliable method for estimation of postmortem submersion interval.

Our best understanding of the process of insect infestation of cadavers is in temperate terrestrial environments (Gennard, 2012; Byrd and Castner, 2010). In these situations after a cadaver is deposited outside, it rapidly attracts adult female flies, typically in the family Calliphoridae, for oviposition. After the eggs hatch and the larvae begin feeding on the fleshy parts of the cadaver a variety of other insects are attracted to the decomposing cadaver. These insects might be additional species of Calliphoridae, Sarcophagidae and flies from other families that intend to lay their eggs on the cadaver. Additionally, predators of fly larvae will be attracted to feed on fly larvae. Some beetles that feed on decomposing flesh or raise their larvae on decomposing flesh are also attracted. Especially in tropical and subtropical environments, ants may also frequently visit a cadaver to feed on the flesh or the insects on the body. These waves of invasion of the cadaver by various species of insects has been referred to as succession but really it is more a continuum of species with particular adaptation to feed on cadavers at different stages of decomposition and invasion by other insect species – a dynamic community. Understanding this continuum of insect infestation of cadavers and the factors that influence this continuum is important in determining the postmortem interval from forensic insect evidence. If the cadaver is fresh and decomposition is not advanced past the initial colonization by one or several species of Calliphoridae, the process of determining postmortem interval is relatively straightforward (Brown *et al.*, 2012; Catts, 1992). If decomposition is more advanced, this estimate is more complicated (Battán Horenstein and Linares, 2011; Catts, 1992). Understanding this continuum requires a series of carefully controlled and monitored experiments using pig carcasses in a variety of environments. Depending on the situation these experiments

may require up to three months to be completed so that the full spectrum of insect succession can be documented. The importance of forensic entomology in determining postmortem interval becomes greater the further along decomposition has progressed as the other postmortem biological indicators become increasingly less precise (Goff, 1993).

Experimental Data

The experimental data necessary for accurate estimation of postmortem interval can be divided into field studies and laboratory studies. The field studies must be carried out in controlled areas so that the pig carcasses are not disturbed by vertebrate scavengers of curious humans and can be easily visited on a regular basis by the investigators. Environmental data must be carefully recorded and the process of decomposition documented photographically. As mentioned previously, these studies must be conducted in a variety of habitats and seasons of the year to get a full picture of the continuum of insect infestation (Brundage *et al.*, 2011; Tabor *et al.*, 2004; Goff, 1993).

Laboratory Studies – Estimation of Postmortem Interval

These field studies will need to be supported by detailed laboratory studies under controlled conditions of temperature. Detailed observations of insect development rate, especially calliphorid and sarcophagid flies are essential for determination of postmortem interval. These experiments must be carefully controlled and conducted under standardized conditions as small variation in conditions such as diet for the larval insects can dramatically alter the results (Rabelo *et al.*, 2011). These studies must be conducted at a variety of temperatures to establish growth rates that can be used to interpret observations from the field (Donovan *et al.*, 2006). In particular, developmental thresholds are necessary to use the accumulated degree-day method to relate insect development to particular temperature regimes (Myburgh *et al.*, 2013; Goff, 1993). These studies will need to be completed for the major fly species that infest cadavers in the various environmental regions of Ecuador. This process has been mathematically modeled (Reibe *et al.*, 2010) and Schoenly (1993) has used a statistical process to analyze these data and put estimates of variance on postmortem intervals. The ability to put rigorous estimates of variability in estimates of postmortem interval is essential to maintaining the reliability of this evidence. These field studies and supporting laboratory experiments represent the core knowledge necessary to begin to use medicolegal entomology

data in crime investigation in Ecuador. This is a significant undertaking that will require years of investigation with financial support to complete.

Specialized Scenarios

After completion of these basic series of observations and experiments, a variety of refinements are possible and desirable to investigate. These additional experiments are typically driven by the needs of crime scene investigators in particular situations that demand additional information. The type of wound that resulted in death may be important in how insects infest a cadaver (Goff, 2000; Byrd and Castner, 2010). Wounds with small entrance apertures (small caliber gunshots, impalement by small sharp instruments) may be less attractive to ovipositing flies than large lacerations caused by slicing wounds and large caliber gunshots with large exit wounds. Similarly large wounds caused by explosions or motor vehicle accidents may attract a different spectrum of insects. These variations in cause of death may be simulated with pigs and the effects on invading insect fauna documented in field experiments. The presence or absence of clothing may also restrict or facilitate access of flies to the cadaver and result in very different patterns of colonization and succession (Voss *et al.*, 2010). Again these effects can be simulated using pigs clothed to variable degrees as appropriate. The use of vehicles to dispose of dead bodies by criminals or the use of closed vehicles for suicide presents significant problems in estimation of post mortem interval as the vehicle restricts access to certain important groups of flies. This can result in a significantly altered continuum of insect infestation and decomposition of the cadaver (Byrd and Castner, 2010). Temperatures in closed vehicles can also be elevated depending on the environmental conditions. Some flies are inhibited from entering buildings so the pattern of insect infestation inside a dwelling or structure may be very different from that on a cadaver outside even within very short distances (Pohjoismäki *et al.*, 2010). All of these scenarios lend themselves to simulation with pig carcasses in appropriate experimental conditions. Burn victims present significant problems as the heat and tissue damage present on the victim may change the attractiveness of the cadaver to insect infestations. Death by fire or the postmortem use of fire to destroy evidence has the effect of drying the cadaver and altering the decomposition process. This in turn alters the insect community that is attracted to the cadaver. Simulation of this process is difficult, as this requires the cooperation of fire fighters and an appropriate structure or vehicle

that can be destroyed for the experiment. In the few instances where this has been done experimentally with pig carcasses, entomological evidence was able to be recovered after the fire (Byrd and Castner, 2010). Simulation of the disposal of a body in fresh water is certainly possible but the resulting environmental contamination usually has made these experiments difficult to perform (Barrios and Wolf, 2011). Studies of this type in the ocean can also be difficult to perform but recent advances in remote video cameras allow long term observation of the decomposition animal carcasses in the ocean from remote locations (Byrd and Castner, 2010). Recently there has been a great interest in the effect of drugs of abuse and recreational drugs in the bodies of crime victims on the development of insects colonizing the cadaver. In some cases development is slowed and in other cases it is accelerated depending on the drug and the concentration in the tissues. These studies are best conducted in the laboratory using diet that has been amended with controlled concentrations of the drugs of interest (Lopes de Carvalho *et al.*, 2012).

Experimental simulation of different crime scene scenarios using pig carcasses is a powerful tool in the development of medicolegal entomology. However, in the end we cannot anticipate every possible crime scene or mode of death. We can only cover the most common or probable situations. Experiential data from collaborations with police, prosecutors and crime scene investigators is essential to the development of forensic entomology. The forensic entomology team needs to be ready to respond rapidly when called upon to go to crime scenes to assist in the investigation and collect data. This is especially true in cases that are unusual or problematic and may be difficult to bring to a successful conclusion.

Homicides and violent crimes against persons that result in death are extremely important and deserve our utmost efforts to resolve but other situations also have relevance to forensic entomology. In many cases the research on insect development and biology on the major insects of forensic importance may crossover to these cases as well. In other cases research must be conducted on other specialized insects as well. Cases involving neglect and abuse of the elderly, children or disabled can also present insect evidence related to the extent of the abuse (Benecke and Lessig, 2001).

Molecular Methods

As methods of DNA analysis become more commonplace in forensic laboratories, these methods will take on greater importance for forensic entomology. Conceivably, larvae and pupae of flies and other insects could be identified rapidly using diagnostic DNA signatures as opposed to lengthy rearing of the fly larvae to adulthood. Females could also be associated with males through DNA sequence information; they currently cannot be identified by morphological characteristics alone. These methods will require a rigorous prepared database to use as reference. Geographical variation in populations of forensically important insects will have to be accounted for. These molecular tools have the potential to be a powerful technique in forensic entomology at all levels.

Preparation and presentation of forensic entomology data

To be truly useful in solving crimes and bringing criminals to justice, forensic entomology must be an active collaboration between entomologists and other biologists, the legal community and the police. Police and crime scene investigators must understand the utility of forensic entomology investigation and evidence. As they are the first responders to a crime scene, it will be their responsibility to collect entomological evidence or call in the entomological rapid response teams for assistance. Especially early in the development of forensic entomology in Ecuador, this collaboration will be essential for the entomological teams to gather biodiversity data and to gain experience with forensically important insects encountered in actual crime scenes.

Prosecutors must actively work with the forensic entomology team and police to develop standards for recording and reporting entomological data. These standards will insure that the evidence collected is acceptable in court proceedings and can be used to arrive at the truth. Judges must be in agreement that entomological evidence is reliable and represents an additional type of evidence that can be important in obtaining a clear picture of the circumstances of a crime. The reliability of entomological evidence established through rigorous research will be the most important factor to convince the legal community that resource should be devoted to forensic entomology.

Entomologists must also be trained by the legal community to present data and evidence using accepted legal standards. This is very different than what we are used to as scientists. This further

underscores the collaborative nature of forensic entomology. We will need to continually work together to establish forensic entomology as a powerful tool in criminal investigation.

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