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Insect succession on remains of human and animals in Shenzhen, China

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ABSTRACT

Most forensic entomological succession studies have been carried out using pig or rabbit carcasses; however, there have been few studies on the differences between insect succession patterns on human cadavers and on animal carcasses. In order to clarify the differences between decomposition and insect succession patterns of human cadavers and animal carcasses, one 49.5 kg human cadaver, two large pig carcasses (45 and 48 kg), two small pig carcasses (23 and 25 kg) and two rabbit carcasses (both 1.75 kg) were placed in the same field conditions in Shenzhen, China for a comparative study on August, 2013. The results indicated that: (1) The duration from fresh to skeletonization is in order of human cadaver > large pig carcasses > small pig carcasses > rabbit carcasses; (2) insect assemblages (including developmental stages) are more complex on larger carcasses, in order of human cadaver = large pig carcasses > small pig carcasses > rabbit carcasses; (3) the developmental rates of the same forensically important fly species on all carcasses are consistent; (4) all identified species of Calliphoridae can complete development of one generation on human cadaver, and both large and small pig carcasses, while on rabbit carcasses, only a subset of the Calliphoridae species can finish development of one generation; (5) beetles can generate offspring on human cadaver, and both large and small pig carcasses, while they do not generate offspring on rabbit carcasses. This study provides useful comparative data for decomposition and insect succession pattern of human cadaver with animal carcasses.

1. Introduction

Investigating insect succession, along with developmental study of carrion insects, constitutes some of the most fundamental and important work of forensic entomology. In the time since insect succession theory on carcasses was first proposed by Mégnin [1], there have been a great number of studies on this field. Through these studies, the succession patterns under different types of environment have been observed, including land exposure [2,3], burying [4,5], indoor environments [6,7], vehicle environments [8], ponds and rivers [9,10], oceans [11], dry environments [12,13], rainy seasons [14,15]. In further studies the effects of other factors have been examined, including the effects of burning [16,17], drugs and toxins [18–20], hanging [21], clothing and covering [22–24], carcass size [24–28], lime [29], penetrating injuries [30], scavengers [31], inter-species competition [32–34], altitude [35], different seasons [36,37], and freezing [38,39].

Research methods in succession studies have been improved continuously, and standards for setting replicates and controls that allow generation of more confident statistics have been proposed [40,41]. There have also been some relevant studies of insect sampling methods [42–44], optimal animal model [45–47], effects of daily sampling [48], the optimal inter-carcass distance [49], the effect of repeated experiments at the same location [50], effects of freezing and thawing [39], and effects of investigator disturbance [51].

Even though forensic entomology has achieved great accomplishments in the studies of succession, unfortunately, most of these studies have drawn conclusions from animal carcasses [43,52,53]. There exist differences between humans and other animals used in studies of succession, for example, humans have longer living spans, walk upright, feed on a wide variety of food sources, wear clothing, have less hair covering the body surface, take different kinds of medicines, take baths frequently and so on. Only in a handful of areas around the world have human cadavers

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been used to conduct studies on forensic insect succession [43,52,54,55], and in this aspect the 'body farm' Anthropological Research Facility at the University of Tennessee is widely renowned [56]. Early in 1983, Rodriguez and Bass [52] used four human cadavers to study insect succession, and by comparing these results with the succession results of 43 dogs by Reed [57] in the same area, they showed that the various insect species observed on human cadavers were found to be the same as those on dog carcasses. A study by Schoenly et al. [43] indicated that carrion insects had negligible preference for human cadavers versus pig carcasses, and argued that pig carcasses weighing about 23–27 kg can substitute for human cadavers for experiments.

Since forensic entomology studies are mainly applied to estimating minimum postmortem interval (PMI_{min}) of humans, the use of human cadavers, if conditions allow, to conduct insect succession studies is the best way to obtain accurate primary data for forensic entomology applications. The insect succession patterns of human cadavers can vary dramatically, especially when we consider the variety of different climatic zones on the earth, thus diverse studies using human cadavers are necessary. Secondly, most forensic entomological succession studies around the world are carried out using pig or rabbit carcasses, however, there is a dearth studies on the potential differences between insect succession on pig and rabbit carcasses and that on human cadavers. Although the theory of 23–27 kg pig carcasses as optimal models has been proposed for a long time and widely accepted, the proposal of this theory also considered factors such as economy and operability; it is not necessarily true that pig carcasses with such weights have exactly identical decomposing patterns and insect succession to human cadavers. Therefore, it is necessary to carry out more comparative studies of decomposition and succession between human cadavers and animal carcasses, and thus facilitate a comprehensive reference for estimating PMI_{min}. Moreover, comparative studies between human and animal remains will aid in development of the discipline of comparative forensic medicine, leading to important advances in understanding, and this will be of benefit to forensic disciplines of both human and animal [58,59].

2. Materials and methods

2.1. Experiment site

This study was carried out in a forest of litchi *Litchi chinensis* Sonn. near the Forensic Autopsy Centre of the Public Security Bureau of Shenzhen City. The litchi plants in the experiment site were used as greening plants rather than fruit trees. The size of this experiment site is approximately 10 hm^2 (500 m long and 200 m wide). There is an artificial lake about 150 m long and 50 m wide in the experiment site, and the litchi forest and artificial grass were planted in a ring around the lake. There are walls with height of about 5 m at the surrounding of the litchi forest. There are factories and residential buildings around the experiment site, so generally the experiment site was in an urban area (urban ecosystem) with frequent anthropogenic activities. The study area is located along the coast of South China (22° 37′ 38″ N, 114° 09′ 30″ E), the altitude is 100 m, and it has a subtropical oceanic monsoon climate with annual mean temperature being 23 °C. The summer in the area is very long and lasts about 6 months, from May to December; the winter is warm, without snow, and with temperature remaining above 0 °C.

2.2. Experimental materials and processing methods

The cadaver of one human male of East Asian descent after postmortem examination in frozen preservation (aged 19 years old, height of 170 cm, weighing 49.5 kg, Hm), two large pig carcasses with weights close to that of the human cadaver: PgL1 and PgL2 weighing 48 and 45 kg, respectively, two small pig carcasses whose weights were close to the recommended weights: PgS1 and PgS2 weighing 23 and 25 kg, respectively; two rabbit carcasses: Rt1 and Rt2 weighing both 1.75 kg were used in this study. The human cadaver was donated by his family and the donation was strictly carried out and registered according to the regulations of the Ethical Committee of Forensic Center. Pigs *Sus scrofa domestica* L. were bought from a commercial piggery and rabbits *Oryctolagus cuniculus* L. were purchased from the Laboratory Animal Center of Southern Medical University. All animal studies were approved by the Institutional Animal Care and Use Committee (IACUC) and carried out under the policies of Soochow University.

Animals were sacrificed on August 26th 2013 by blunt blow to the head at 8:00 to 9:00 am. The human cadaver was thawed 12 h in advance in a closed room without insects. All the carcasses were double-packaged with black plastic bags and transported to experiment field from 9:00 to 10:00 am. The litchi trees are sparse, and the animal carcasses were placed in the open area within the woodland where the sunlight could directly shine on the carcasses. The vegetation around the animal carcasses consisted mainly of wild weeds. The experiment site was maintained so that the weeds in the litchi forest were limited. The human cadaver was placed next to the Forensic Autopsy Centre where the litchi trees meet the lawn, in order to facilitate our management. The vegetation around the human cadaver was mainly artificial grass. The carcasses were placed with a north-south direction, and the inter-carcass distance was $\geq$50 m. A metal cage (2 m × 1.5 m × 0.8 m) was placed on carcasses in order to keep them from being eaten by scavengers. Iron fences with a height of 4.5 m were built around the human cadaver to avoid disturbance.

2.3. Investigation protocol

This study started on August 26th, 2013 and ended on December 21th, 2013. On the exact day of placing carcasses, observation was conducted every two hours, and after that observation was conducted twice every day at 9:00 am and 3:00 pm. After the carcasses became skeletonized, observation was carried out once at 9:00 am every day. Insects were collected until no forensically relevant taxa were present. When there was no forensically relevant taxon on carcasses, the investigation was concluded. Field investigation tools were used as per the recommendation of Amendt et al. [42].

The degree of carcass decay was observed, recorded, photographed and videoed. The degree of decay of the three parts of carcass, i.e., head/neck, trunk, limbs were scored, according to the system proposed by Megyesi et al. [60]. The scores of each part were added together to get the total body score (TBS) (from a minimum of 3 to a maximum of 35 points), representing the decay degree of the total carcass. Two Testo l75-H1 temperature and humidity recorders (Germany) were equipped 1.2 m above the land surface in places sheltered from sun and rain, and temperature and humidity were recorded automatically every hour.

Insect sampling was conducted along with observation. Samples were collected manually, and the sampling objectives included eggs, larvae, pupae, adults and puparia. The observing and sampling areas included above and beneath carcasses and above and below ground within the radius of 10 m surrounding carcasses. During the sampling process, the impact on insects was minimized as far as possible. For beetles that were easy to identify and were few in number, observation was conducted without sampling. For insects above the ground, observation and sampling were conducted directly. For sampling of insects distributed inside