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Annual variation in decomposition and insect succession at a periurban area of central Iberian Peninsula

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ABSTRACT

The variation in decomposition and insect succession among the four seasons of one year was studied for the first time in a periurban area of central Spain. During the winter trial, the carcasses showed corification, a cadaveric preservation phenomenon which apparently leaded to a significant delay in decomposition processes. The composition of the insect fauna breeding on carcasses changed significantly between trials. Active decay was mainly driven by Calliphoridae (Diptera) larvae in every season except in winter trial, when larvae of *Thanatophilus* species (Coleoptera: Silphidae) were the main consumers of soft tissues. Advanced decay was characterized by the occurrence of Dermestidae, Silphidae, Cleridae, Nitidulidae (Coleoptera) and Piophilidae (Diptera) larvae. Differences in the species composition in comparison with other regions of the Iberian Peninsula were also observed. The current paper provides baseline and preliminary information on the insect succession on carrion in central Spain, as well as a starting point for further research on forensic entomology in this region.

1. Introduction

Forensic entomology, the analysis of insect evidence to aid in legal and forensic investigations, has become one of the most reliable tools for the estimation of the minimum postmortem interval (PMI_{min}).[1] The entomological methods for this estimation have increased significantly in number, availability and accuracy during the last decade, up to the point that they are currently a common and reliable practice throughout the world. Research on forensic entomology grows exponentially, covering a wide range of topics such as insect succession on carcasses, developmental models, molecular analyses, or entomotoxicology.[2] However, a lot of work still needs to be done and the precision and accuracy of the methods applied are continuously reviewed and discussed.[3–6]

The predictability of the arthropod succession on carrion and its potential use to estimate the PMI_{min} are central concepts in forensic entomology.[4] Whereas the PMI_{min} can be reliably estimated in the first weeks after death with the use of development time data for forensically relevant species, successional data are useful for longer periods of time.[1] As the composition of species associated with carrion varies among seasons and geographical regions,[7,8] successional data can provide important insights into an investigation in long PMI cases, e.g. helping to identify the season of the year when death occurred, or indicating the postmortem relocation of a corpse.[1] Hence, generating PMI_{min} estimates with the use of successional data requires baseline data specific for a given region and season.[4] In this sense, despite numerous succession studies have been published throughout the world, some geographical areas remain poorly studied.

A clear example of understudied area is the Mediterranean region and, more concretely, the Iberian Peninsula. Due to its geographical situation, the Iberian Peninsula is influenced by both the Atlantic Ocean and the African arid air masses, showing a high climatic diversity.[9] This climatic heterogeneity entails in turn significant differences in the decomposition patterns of corpses between coastal and interior provinces.[10] Moreover, preliminary studies from mid-20th century[11,12] already suggested differences in the composition and succession patterns of the necrophagous insect fauna between different areas of the Iberian Peninsula. Recent studies in western,[13,14] northeastern,[15] eastern[16] and southeastern[17,18] Iberian Peninsula have certainly revealed differences in that sense. Furthermore, recent ecological studies carried out in the peninsular centre[19,20] showed not only differences in the composition of the fauna with respect to other Iberian regions, but also important differences between seasons within the same areas. Unlike the coastal and peripheral areas, the centre of the Iberian

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Peninsula shows highly extreme temperatures throughout the year, with very cold winters and very hot and dry summers.[9] The central region also shows the highest population density of the Iberian Peninsula, and therefore the highest proportion of suspicious deaths. However, there is a lack of successional data from this region. The only published succession study[21] was a preliminary experiment on the insect succession on piglet carcasses carried out during a single spring season. Hence, there is an urgent need for baseline successional data from the centre of the Iberian Peninsula and from the different seasons.

The aim of the current paper is to provide baseline and preliminary information on the insect succession on carrion in the centre of the Iberian Peninsula, in order to (i) determine the decomposition and succession patterns of the insect fauna associated with decomposing remains in a periurban area of that region, and (ii) analyze the variation in those decomposition and succession patterns between the four seasons of one year. Whereas the current research is preliminary and its results cannot be taken as a predictive model until the experiment has been replicated during several years, it provides reference data which will contribute to fill the existing gap in the study area.

2. Materials and methods

2.1. Site description

The experiment was carried out in a private property belonging to the University of Alcalá, Madrid Community, central Spain. The study site (40° 30′ 44.54″ N, 3° 19′ 41.58″) is an uncultivated plot of 30 Ha with dominant ruderal vegetation, at the fringe of an urban environment. It is located on the Mesomediterranean bioclimatic level, the most extended in the Iberian Peninsula,[9] at an elevation of 650 m a.s.l. The annual average temperature of the study site is 13–17 °C and the annual average rainfall is about 450 mm.

2.2. Animal model

We used 12 domestic pig (*Sus domesticus* Erxleben) carcasses of similar weight (range = 24–32 kg); 3 carcasses for each season: summer, autumn, winter and spring. Pigs were killed by sodium phentobarbital overdose in the Centre for Research Support of the University of Alcalá, in accordance with the European Union's Council Directive 86/609/EEC. The carcasses were immediately transferred in plastic bags to the study site. The date and hour of placement of the carcasses for each season trial were as follows: 28th June 2004, 15:45 h (summer); 21th September 2004, 15:45 h (autumn); 11th January 2005, 12:15 h (winter); 4th April 2005, 13:00 h (spring). The carcasses were placed on individual mesh platforms with approximately 60 m of separation[3,6] and protected with wire mesh cages to avoid the action of vertebrate scavengers but allowing the access of insects.

2.3. Sampling procedure

Each carcass was examined during one year; three carcasses per season trial: summer trial from June 2004 to July 2005; autumn trial from September 2004 to October 2005; winter trial from January 2005 to February 2006; and spring trial from April 2005 to May 2006. From the three carcasses used in each trial, one served as control from which no insects were collected and two represented experimental replicates.

Carcasses were examined daily until the end of active decay; then, on alternate days until the end of the respective trial season; and then, weekly until the completion of the first year of survey. The inspection of each carcass was always performed by two researchers and lasted about 30 min. First, flying insects were collected with an entomological net. Secondly, the insects on, inside and below the carcass were collected using forceps or a brush, including representative samples from each egg cluster and larval mass. Finally, the soil below and around the carcass was dug and stirred to collect adult and immature insects. Once at the laboratory, immature stages of Diptera were placed into incubators under controlled temperature and photoperiod (25 °C and 16:8 L:D) and reared until adult emergence. Both sampled and reared adults were killed by freezing and subsequently identified. For Coleoptera immatures, 50% of specimens were reared in the same conditions for identification, and the remaining 50% were conserved in 70% ethanol. Specimens have been deposited in the collection of the Department of Life Sciences of the University of Alcalá.

Every sampling day, each control and experimental carcass was weighed by two persons hanging the mesh platform with the carcass from a bar with a dynamometer KERN CH50K50. Regrettably, weight records from the control carcass in summer trial are not available and therefore they are not included in the current study. The state of the carcasses was classified within its correspondent stage or process of decomposition in accordance with the definitions suggested by Matuszewski et al.,[22] and documented by means of both photographs and written reports containing all the relevant information. Air temperature, relative humidity and precipitation were recorded hourly throughout the whole experiment using a weather station (Mobile Weather Station μMETOS®) located about 1 km from the study site. During the inspection of the carcasses, air temperature was confirmed on site using a digital hand thermometer (Digi-Sense, Cole-Parmer® type J Thermocouple Md 8528–30).

The Jaccard Similarity Index (JSI) was used to compare the similarity of the immature carrion insect composition among carcasses. JSI is a measure of beta diversity based on presence or abundance data (i.e. independent of sample size[23,24]), commonly used in forensically focused succession studies.[6,25] The similarity among carcasses according to JSI values was visualized by means of a cluster analysis. We considered only those Diptera and Coleoptera species collected as immature specimens either feeding on the dead tissues or preying on other necrophagous insect larvae. Immature individuals reside in and around the carcass, whereas insect adults are highly mobile[6]; therefore, the presence/absence of insect species collected only as adult specimens may be a potential source of cross-contamination between experimental carcasses.[3,5,6] The analyses were performed using PAST 3. x.[26]

3. Results

Fig. 1a shows minimum, maximum and average daily temperature and precipitation during the four seasons of study. As expected in a Mediterranean continental area, summer was hot and dry, whereas winter was typified by low temperatures and by a precipitation decrease in comparison to autumn (Fig. 1a).

Four stages of decomposition were recorded in every carcass: fresh, bloating, active decay and advanced decay. In both summer and autumn trials, the four stages were observed in that consecutive order, with a short duration of both fresh and bloating stages (Table 1). However, in both winter and spring trials, the onset of the active decay preceded the onset of the bloating stage, with both processes overlapping in time (Table 1). It must be noted that some overlap occurred also in summer, as the first larval masses (which marked the onset of the active decay) were observed one day before the end of the bloating stage (Table 1). On the other hand, the decomposition of the carcasses from winter trial was significantly retarded, as they were frozen during several weeks due to the extremely low temperatures (Fig. 1a), and the first larval masses were not observed until 30–31 days post-mortem (Table 1). This slowdown of the decomposition during winter trial was reflected on the lower rates of weight loss of the carcasses (Fig. 1b). Accordingly, in winter carcasses the bloating and active decay processes started later and lasted for longer in comparison to the other season trials (Table 1). Consequently, the advanced decay did not start until days 100–109 in winter carcasses (Table 1), thus running during the subsequent spring season. Furthermore, winter carcasses showed the cadaveric preservation phenomenon called corification or leathery, in which the skin resembles elastic and greasy tanned leather.[27]