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CASE REPORT

Post-mortem Interval Estimation in a Forensic Case with Two Predatory Species: *Chrysomya albiceps* and *Synthesiomyia nudiseta*

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ABSTRACT

A forensic case in which two facultative predatory species were found together on a human corpse is presented in this work. Second and third instar larvae of *Synthesiomyia nudiseta* (van der Wulp, 1883) (Diptera: Muscidae) and *Chrysomya albiceps* (Wiedemann, 1819) (Diptera: Calliphoridae) were collected from the dead body of a man on 14th November 2019, but puparia in the autopsy pictures were observed after, but not collected. The corpse was found on the terrace of the third floor of a building located in the town of Elche (SE Spain), and the pathologist confirmed the death as natural. The minimum post-mortem interval was estimated by the time to reach the length of the collected larvae or to reach the pupal developmental stage (in days and accumulated degree-days). In accordance with the influence of the predatory behaviour of each of these species on the developmental data of the other, as described in the existing literature, the effect of a hypothetical competition between both species on post-mortem interval was discussed.

KEYWORDS: forensic entomology, post-mortem interval, predatory behaviour, *Chrysomya albiceps*, *Synthesiomyia nudiseta*, Spain

INTRODUCTION

There are many different interactions between the fauna on a corpse, such as competition between or predation on the larvae of necrophagous species of Diptera [1–3]. Competition is more common than predation [4]; but when these two phenomena occur at the same time, that is to say where a predator kills and eats its competitors, this process is called intraguild predation (IGP) [3]. IGP can take place between individuals of the same species, or between individuals of different species; and on carcasses, IGP should be taken into account in the estimation of post-mortem interval based on the entomological evidence, but at present few reports about forensic cases of this nature have been published.

Besides being forensic indicators, *Synthesiomyia nudiseta* (van der Wulp, 1883) (Diptera: Muscidae) and *Chrysomya albiceps* (Wiedemann, 1819) (Diptera: Calliphoridae) are facultative predatory species during their last larval instar [5–7]. Both species are forensic indicators in southwest Europe, being early colonizers in autumn, *C. albiceps* peaking in September and *S. nudiseta* in November [8]. The predatory behaviour by *C. albiceps* is well-known [6, 9–11], but it is not the case of *S. nudiseta*; however, the predatory behaviour on other Diptera larvae of this muscid was recently confirmed. Ivorra et al. [5] studied the combination of these species in the laboratory at the same time and trophic medium, and the rate of predation by *S. nudiseta* was found to be higher than that by *C. albiceps*; more recently, the prey-predator interaction between them without food was studied by Barbosa et al. [12]. However, it is important to know how the interaction between these species on human remains could affect the post-mortem interval estimation. So, due to the importance of knowing the predatory behaviour of these species in estimating the date of

death in forensic cases, a forensic case in Spain in which these both species coexisted is reported, and the effect of their predation ability on the estimation of minimum post-mortem interval (mPMI) is discussed.

CASE PRESENTATION

The body of a man was found on 14th November 2019, on the outside rooftop terrace of his home situated on the third floor of a building located in Elche, province of Alicante, Spain. The corpse was clothed and was located in a supine position on the ground. The autopsy was performed at the Legal Medicine Institute of Alicante (IMLA) on the day after discovery of the corpse, at 10.00am; the corpse had been kept into a body freezer (-5ºC) at the morgue for approximately 12 hours beforehand. According to the pathologist's examination, the death was natural, due to his previous cardiovascular and respiratory pathologies, and was estimated to have occurred 15 - 21 days before the discovery (25th to 31st October 2019). The body was in bloated stage. The meteorological data from the nearest weather station, situated at 2.5 km distance away from the discovery scene, were provided by the State Meteorological Agency (AEMET). The daily minimum, maximum and mean temperatures and humidity for the 30 days before discovery of the body (16th October to 14th November 2019) were recorded; the average of these daily minimum (12.1 ± 2.9ºC), maximum (23.4 ± 3.9ºC) and mean temperatures (17.2 ± 3.1ºC) were obtained; the average relative humidity was 61.9 ± 12.7%.

Entomological Evidence

Table 1 shows the entomological evidence sampled during the autopsy by the pathologist at the IMLA. Specimens were transported to the laboratory of the Department of Environmental Sciences and Natural Resources in the University of Alicante, where the larvae were identified by the taxonomic key of Velásquez et al. [13] using a stereo microscope (Leica M80). Moreover, photographs of the autopsy and data about the death scene were provided from IMLA. Second and third instar larvae of *Synthesiomyia nudiseta* and *Chrysomya albiceps* were collected from the larval masses situated on the neck and in the centre of the thorax (Figure 1). However, on examining the autopsy photographs, puparia on clothes were observed, but they could not be identified in the laboratory (Figure 2). All puparia observed in the photographs, which were not sampled, appear complete (i.e., prior to adult emergence) and similar to *S. nudiseta*, but it is not possible to discount the possibility that there could have been empty puparia or puparia of both species at the scene.

Table 1 Entomological samples collected from the corpse during the autopsy and preserved in 70% alcohol (LII, second instar larvae; LIII, third instar larvae).

Taxa	Species	Developmental stage	Maximum size
Calliphoridae	*Chrysomya albiceps*	LII*, LIII**	15.6 mm
Muscidae	*Synthesiomyia nudiseta*	LII*, LIII*	18.2 mm

Asterisk indicates the number of specimens collected and analysed *1–20; **>100.

Estimation of the minPMI

Growth Curves

The maximum lengths of the larvae collected were 18.2 mm for *S. nudiseta* and 15.6 mm for *C. albiceps* (Table 1). Since there is no published growth curve for *S. nudiseta* at 17ºC, we used instead published growth curves for 20ºC and 15ºC, at which temperatures to reach 18.2 mm *S. nudiseta* requires 7 days and 13 days respectively [14]. There is no growth curve of *C. albiceps* at 15ºC, so attending to its curve at 20ºC, this species needs 10.5 days, three more than *S. nudiseta* at the same temperature [15], and according to these authors, both species reach these lengths just before stopping feeding (prepupa). In this first approximation, the estimation of the minPMI was from the 2th to 8th November based on *S. nudiseta* at 15ºC and 20ºC respectively; and 5th November based on *C. albiceps* at 20ºC (Table 2). However, we should take into account that the temperatures used for these estimations are 2ºC lower (using 15ºC) and 3ºC higher (using 20ºC), than the actual 17ºC mean temperature of the scene. Therefore, *S. nudiseta* would be expected to develop faster and *C. albiceps* slower than our estimations.

Figure 1 Corpse of a man after removal of clothing in a bloated stage during autopsy

Figure 2 The pupae found on the clothed of the body (red circle and arrow)

Table 2 Estimation of the minimum post-mortem interval with accumulated degree-days with *Chrysomya albiceps* and *Synthesiomyia nudiseta* (t = daily mean temperature; t_0 = minimum threshold temperature; DD = degree-days; ADD = accumulated degree-days)

			Synthesiomyia nudiseta			*Chrysomya albiceps*		
Date	Day	t	t_0	DD (t - t_0)	ADD	t_0	DD (t - t_0)	ADD
14/11/2019	1	11.1	3	8.06	8.06	10.2	0.86	0.86
13/11/2019	2	16.8	3	13.78	21.84	10.2	6.58	7.44
12/11/2019	3	14.5	3	11.47	33.31	10.2	4.27	11.71
11/11/2019	4	12.9	3	9.88	43.19	10.2	2.68	14.39
10/11/2019	5	14.0	3	10.98	54.17	10.2	3.78	18.17
09/11/2019	6	14.7	3	11.72	65.89	10.2	4.52	22.69
08/11/2019	7	14.0	3	10.99	76.87	10.2	3.79	26.47
07/11/2019	8	15.5	3	12.48	89.35	10.2	5.28	31.75
06/11/2019	9	16.1	3	13.11	102.47	10.2	5.91	37.67
05/11/2019	10	17.7	3	14.70	117.17	10.2	7.50	45.17
04/11/2019	11	21.2	3	18.24	135.41	10.2	11.04	56.21
03/11/2019	12	21.2	3	18.21	153.62	10.2	11.01	67.22
02/11/2019	13	21.7	3	18.69	172.31	10.2	11.49	78.71
01/11/2019	14	22.4	3	19.35	191.66	10.2	12.15	90.86
31/10/2019	15	22.0	3	18.95	210.61	10.2	11.75	102.61
30/10/2019	16	20.6	3	17.58	228.19	10.2	10.38	112.99
29/10/2019	17	19.8	3	16.80	244.99	10.2	9.60	122.59
28/10/2019	18	16.2	3	13.25	258.24	10.2	6.05	128.64
27/10/2019	19	17.0	3	14.02	272.26	10.2	6.82	135.46
26/10/2019	20	17.0	3	13.98	286.24	10.2	6.78	142.24

Duration of developmental stages and ADDs

On the basis of the fact that there were some unidentified puparia, the period for the time to reach the pupal stage was included in the analysis. The developmental time for *S. nudiseta* from egg to pupariation is between 10.56 days at 20ºC and 17.33 days at 15ºC [14]. Regarding *C. albiceps*, Grassberger et al. [15] observed that the larvae failed to develop beyond the first instar at 15ºC and, at 20ºC, they established a minimum developmental time from egg to pupariation of 12.9 ± 0.41 days. On the basis of *S. nudiseta*, it is possible that the corpse was exposed to this species between 29th October to 5th November, in base to temperatures of development (15ºC and 20ºC) used (Table 2). In the case of *C. albiceps*, eggs could have been laid on 2nd November, three days before than *S. nudiseta* at the same temperature (20ºC).

Regarding Accumulated Degree-Days (ADD), for *S. nudiseta* the egg and total larval period (i.e. from egg to pupa) is 179.52 ADD at 20ºC (10.6 days) and 207.96 ADD at 15ºC (17.3 days), using a lower development threshold of 3°C [14]. Taking into account that the daily average temperature for 30 days before discovery was 17.2 and the daily average temperature each day before autopsy (Table 2), the retrospective ADDs were calculated, and the result indicates that *S. nudiseta* laid eggs on the body between 31st October and 1st November. In the case of *C. albiceps*, with the data of Marchenko [16], 123.00 ADD were obtained from egg to pupariation at 17ºC, with a 10.2ºC lower threshold, so the corpse was exposed to its activity 18.1 days before autopsy, and attending to ADDs, the value 123.00 ADD is obtained on 28th October (Table 2). These results are compatible to what was observed in the previous analysis using measurements or development stages, so we concluded that the ranges of the two species overlap.

DISCUSSION

Two facultative predatory species were found in this case report from the southeast of Spain. Their predatory ability had been studied before and the results showed that *Synthesiomyia nudiseta* was a more active predatory species than *Chrysomya albiceps* [5]. The mortality of the larvae of *C. albiceps* was greater than that of *S. nudiseta* in mixed and pure cultures at all densities, reaching 100% when they competed against each other at high densities. The study in question was performed under laboratory conditions, but it can still allow us to better understand the application of this factor in a real forensic case in which the two species competed on the same corpse. Recently, Barbosa et al. [12] found that S. *nudiseta* had a higher survival compared with other prey species facing *C. albiceps* due to its strong defensive response that enabled it to counter-attack the predation.

Regarding to the studied case here, a preliminary analysis of the larval samples on the corpse indicated a higher abundance of *C. albiceps*. However, on the clothes in the autopsy photographs, some puparium of *S. nudiseta* were observed, but not of *C. albiceps*. Due to the impossibility of determining which species arrived first and the impossibility of determining which species colonised the body in greater abundance, developmental data for both species were used to estimate the minPMI. If we use the length of the specimens found on the corpse, we can establish a minPMI between 7-13 days for *S. nudiseta* (at 20ºC and 15ºC respectively); and 10.5 days for *C. albiceps* (at 20ºC, because no pupae are obtained at 15ºC based on literature) [14, 15]. Attending to the developmental data up to the pupal stage and the calculated ADDs of the muscid *S. nudiseta*, the minPMI comes out at 10-17 days (so between 29th October to 5th November), which is shorter than the period considered by the pathologist (25th October to 31st October). On the other hand, if we use the data of *C. albiceps*, a minimum value of around 18 days can be established (28th October). These dates are compatible with the date established by the forensic pathologist in this case. However, due to the lack of collected all samples in this case, we will hypothesize different scenarios for taking into account the coexistence of both species.

Intraguild competition affects the size, the developmental time and the mortality of both species [5, 17]. When they are together on the same food resource, the developmental time is longer than when the species are alone: 2-4 days more in *C. albiceps* and less than 2 days in the case of *S. nudiseta* at all densities. In addition, the mortality is higher in *Chrysomya albiceps* when sharing food with *Synthesiomyia nudiseta*, almost reaching 100% at higher densities [5]. However, with regards to size, the presence of both predatory species allows them to reach a greater size than when in pure cultures [1, 5]. Applying these results to our case report leads us to think that if the developmental time of *C. albiceps* is longer on the corpse, the period between the maximum and minimum values of mPMI should be shorter when both species are present. However, in this case, the number of larvae of *C. albiceps* (*n*=121) collected was greater than for *S. nudiseta* (*n*=7), so different options arise.

The first possibility that we hypothesize, which is not consistent with the study of Ivorra et al. [5], is that both species had similar larval abundances in the corpse, but the predatory ability of *C. albiceps* was greater than that of *S. nudiseta*. However, the size of the larvae found on the corpse contradicts this hypothesis, as the greatest size was found in *S. nudiseta* (18.2 mm). As previous studies have established, this shows that the presence of *C. albiceps* on the corpse benefits the muscid, with it being able to reach a greater length than when *S. nudiseta* is without competition [1, 5]. Also, Barbosa et al. [12] found that *C. albiceps* did not predate on *S. nudiseta* due to its counter-attack behaviour when they shared the same space in low and intermediate densities.

The second option concerns the temperature, as ten days before the discovery (from day 1 to day 10), the mean temperature dropped to 14.7ºC. At this temperature, *S. nudiseta* can complete its development without any problem, as previous studies have confirmed [14], but this is not the case of *C. albiceps*, which is not able to complete its life cycle at less than 15ºC [15]. Nevertheless, a large number of *C. albiceps* larvae were found on the corpse, so we hypothesize that its developmental time was longer than *S. nudiseta*, as we can confirm with the previous literature: Marchenko [16] estimated 25.6 days from egg to pupariation at

15ºC for *C. albiceps*, while Velásquez et al. [14] obtained 17.3 days from egg to pupariation for *S. nudiseta* at the same temperature. Although the temperature of the larval mass was not taken into account due to the impossibility to access the autopsy, the high larvae number of *C. albiceps* compared with *S. nudiseta* larvae number in the corpse, and the presence of puparia of *S. nudiseta*, allow us to think that both species did not interact so there are not competition between them [12]; so, *C. albiceps* could develop in the body without problems. The high density of *C. albiceps* could increase the larval mass temperature [18, 19], and due to the mean temperature was around 20ºC in the previous days to autopsy (from day 11 to day 20; table 2), the eggs hatched satisfactory; so only the last instar could be affected by the temperature, slowing down *C. albiceps* development.

Keeping in mind that *S. nudiseta* is commonly known as an indoor species and found in close proximity to human environments, and that *C. albiceps* does not have a strictly endophilic character, the third hypothesis in this case report is that *S. nudiseta* arrived before *C. albiceps*. Thus, the development of *S. nudiseta* started earlier and was faster, and this is the reason for finding fewer of its larvae on the corpse and its pupae in the clothes. Also, Ivorra et al. [5] established that the developmental time of *C. albiceps* is almost 4 days longer when it is with *S. nudiseta* than when it is alone. In fact, *S. nudiseta* could have reached pupariation, and due to the fact that puparia of this muscid are generally found on the corpse, they can be collected during the autopsy. We could not collect entomological evidence during the autopsy or at the location of the corpse, and the forensic pathologist overlooked the puparia that clearly were present. Despite this, we observed on autopsy photographs some puparia attached to the clothes with the characteristic sticky substance of *S. nudiseta* (Figure 2).

The last possibility that we hypothesize is that both species laid eggs on the corpse at the same time. This hypothesis is supported by all the factors in intraguild competition that can affect the usual development of the two species. If we use the ADDs for *S. nudiseta*, the muscid found the corpse at some time between 31st October and 1st November, and its larval length establishes a minimum value for the post-mortem interval of 7-13 days (2th to 8th November). With the ADDs of *C. albiceps*, we can deduce that the blow fly found the corpse at some time on 28th October (18 days). However, if we take into account that both species could have arrived at the same time, and the development of *C. albiceps* is delayed by 4 days when it is with *S. nudiseta*, as Ivorra et al. [5] reported, *C. albiceps* larvae would be 14 days old, and arrived on the corpse at some time on 1st November. Therefore, the mPMI can be established in this case as being 14 days; the greater larval length of both species than they are presented alone and the possible delay in the development of *C. albiceps*, based in Ivorra et al. [5] study, support this hypothesis.

CONCLUSION

In conclusion, there are a lot of factors which should be taken into account when the mPMI is estimated. Intraguild competition is one of them, and the forensic entomologist should keep this in mind when entomological evidence is analysed, even when the larvae do not display predatory behaviour. However, more studies are needed on this topic [5, 12]. Another important factor is the protocol for collecting forensic evidence: the collection of different types of samples, such as larvae with different morphotypes and specimens from different places, during the autopsy, and at the death scene is crucial in order to avoid losing information of forensic value [20, 21]. However, in many countries such as Spain, police and forensic pathologists belong to different government administrations and the information regarding a forensic case is obtained independently (police at the death scene and pathologist during the autopsy) [22], so not all the entomological evidence and case data are recorded. More training for both administrations involved in forensic cases is needed to collect evidences to the same standard. Another critical factor and not always easy to obtain is the temperature at the death scene or the larval mass temperature [18, 19]. It is crucial to provide a good training to the police and pathologist in forensic entomology, as well as to follow the protocols when a dead body is found to collect properly the entomological evidences, to get the accurate temperature and the rest of discovery scene data [20, 21], as all these aspects will allow us to establish a more accurate estimate of the mPMI.

Conflicts of Interest

Authors declare none.

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Author Contributions

All authors contributed to the ideas and design of the article. The literature search, data collection and analysis were performed by Tania Ivorra, Salvador Giner and Anabel Martínez-Sánchez. The first draft of the manuscript was written by Tania Ivorra. All authors commented on the various versions of the manuscript and critically revised the work. All authors have read and agreed to the published version of the manuscript.

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