Pest Technology ©2011 Global Science Books

A Preliminary Analysis of Early Colonization of Pig Carcasses by Blowflies (Diptera: Calliphoridae) in the Iberian Peninsula

Yelitza Velásquez* • **Anabel Martínez-Sánchez** • **Santos Rojo**

Department of Environmental Sciences and Natural Resources / Biodiversity Research Institute CIBIO. University of Alicante, Apdo. 99. 03080 Alicante, Spain

Corresponding author: * **yelitza.velasquez@gmail.com**

ABSTRACT

An important goal in forensic entomology is the identification of necrophagous species that breed directly on corpses. In the Iberian Peninsula (Southwest Europe), there are few studies on carrion-breeding blowflies using pig carcasses as a model for study. In this work, we compared Diptera colonization in the early stages of decomposition in autumn and winter. For each season, a domestic pig (*Sus scrofa*) weighing approximately 20 Kg was used. The carcass was exposed in a semi-urban area at the Campus of the University of Alicante (Southeast Spain), for 10 days. After this period, the pig was covered with a net in order to avoid further insect colonization, whilst allowing the development of species that had already begun to breed under natural conditions. Several samples of immature stages were collected daily and reared under controlled conditions (23°C, 60-70% RH and 14:10 L: D photoperiod). The results showed different carrion-breeding species for the two seasons. In autumn, we found *Lucilia sericata*, *Chrysomya albiceps*, *Chrysomya megacephala*, *Calliphora vicina* and *Calliphora vomitoria*. However, in winter, only *Calliphora vicina* and *Calliphora vomitoria* emerged. *Chrysomya albiceps* was dominant in autumn and *C. vicina* in winter. Natural openings of the head (eyes, nostrils, mouth and ears) were the first sites to be colonized, followed by the anus. This study constitutes the first record of *Chrysomya megacephala* developing in natural conditions in Europe, confirming the forensic importance of the oriental latrine fly in Europe.

Keywords: early decomposition stage, forensic entomology, sarcosaprophagous flies, southern Europe

INTRODUCTION

Blowflies are the first insects to discover and colonize human remains. They are found throughout the world and, along with flesh flies (Sarcophagidae) and other sarcosaprophagous flies, provide useful information related to the estimation of postmortem interval (PMI). Their larvae feed and develop on carcasses, therefore, based upon the life cycle stages of particular fly species recovered from a corpse, or from the succession of insects present on a body, it could be possible to estimate the time of death (Erzinçlioğlu 1996; Byrd and Castner 2010).

In the Iberian Peninsula (SW Europe), there are some studies on species composition and ecology of local necrophagous flies (Martínez-Sánchez *et al.* 1998; Martínez-Sánchez *et al.* 2000a, 2000b, 2002; Martínez-Sánchez 2003; Romera *et al.* 2003; Martínez-Sánchez *et al.* 2005). However, studies of the blowfly community related to the decomposition of animal remains are scarce. In Spain, Castillo (2002) evaluated the succession of entomofauna on pig carcasses in the province of Huesca (N Spain) and Arnaldos *et al*. (2001, 2004) studied succession on chicken carcasses in the province of Murcia (SE Spain). In Portugal, Prado e Castro *et al*. (2008) give information about the succession patterns of Calliphoridae on pig carcasses.

The Iberian Peninsula covers a wide diversity of habitats, which makes it necessary to evaluate the entomofauna composition within specific environments. The generation of these data will be beneficial to forensic investigation in the Mediterranean region. We focus our study on true carrion-breeding species and as a first approximation we centre our attention on the early stages of decomposition. This is considered to be a crucial stage, when insects begin the invasion of the body, as long as there is no delay in access to the body (e.g., burial, wrapping or concealment of the body). Furthermore, the first species to arrive are those used to estimate the postmortem interval (PMI) during the first weeks after death. Hence, the aim of this study was to evaluate the first wave of blowflies breeding in a pig carcass during the first 10 days of decomposition in the four seasons of the year. In this paper only results from autumn and winter are presented.

METHODS

The study was carried out at the Campus of the University of Alicante (38° 22′ N, 0° 31′ W) (Alicante province, SE Spain) in an open semi-urban area, exposed directly to sunlight. The vegetation was dominated by pines, *Pinus halepensis* (Miller 1768), *Acacia* (Miller 1754) and palms, *Phoenix canariensis* (Chabaud 1882). The experiment was undertaken in autumn (November 2007) and winter (February 2008). The averages of minimum and maximum temperatures and relative humidity recorded were 8.6°C, 32.8°C, 65.7% and 6.0°C, 18.9°C, 69.3%, respectively in autumn and winter.

For each season, a domestic pig (*Sus scrofa* Linnaeus, 1758) weighing approximately 20 Kg was used. They were killed by contusion and bagged to prevent non-controlled colonization until the experiment was initiated eight hours after death. The carcass was placed within a metal cage (100 × 100 × 100 cm) to exclude vertebrate scavengers and was exposed for ten days. After that, it was completely covered with an ultra-fine mesh (0.6 × 0.6 mm) to avoid further insect colonization, but allow the development of the larval community that had already been breeding under natural outdoor conditions.

Samples of larvae and pupae were collected manually at 24-h intervals. The eggs were not manipulated to avoid disturbing the initial natural colonization. Larvae were collected during the first 15 days and reared to adult stage under controlled conditions. They were separated according to morphotype and placed in a plastic jar (21 cm diameter × 10 cm deep) with pig liver *ad libitum*, a thick layer of sawdust at the bottom (≈5 cm) and covered with a

Received: 28 January, 2010. Accepted: 10 July, 2011.

Original Research Paper

Table 1 Absolute and relative abundance for each species, including larvae reared under controlled and outdoor conditions.

Species	Autumn			Winter		
	Controlled (23ºC)	Outdoor (mean±SD) (17.3ºC ± 8.2ºC)	Total	Controlled (23ºC)	Outdoor (mean±SD) (11.7ºC ± 4.7ºC)	Total
C. vicina	1003 (17.36)	364 (3.83)	1367 (8.95)	1441 (98.0)	1987 (95.2)	3428 (96.3)
C. vomitoria	8 (0.14)	4 (0.04)	12 (0.08)	30 (2.0)	101 (4.8)	131 (3.7)
Ch. albiceps	1127 (19.51)	8231 (86.51)	9358 (61.26)	0	0	0
Ch. megacephala	13 (0.22)	40 (0.42)	53 (0.35)	0	0	0
L. sericata	3628 (62.77)	858 (9.02)	4486 (29.37)	0	0	0
Total (100%)	5779	9497	15276	1471	2088	3559

net. The jars were placed in an environmental chamber at 23°C, with 60-70% RH and a photoperiod of 14: 10 (L: D). All pupae obtained from larvae reared on the carcass under natural conditions were separated daily and kept outdoors in net cages until adults emerged. Finally, all adults were identified according to González-Mora (1989), González-Mora and Peris (1988), Peris and González-Mora (1991), Rognes (1994) and Rognes and Paterson (2005).

RESULTS

Autumn

The first flies that colonized the pig carcass in autumn were: *Lucilia sericata* (Meigen, 1826), *Calliphora vicina* Robineau-Desvoidy, 1830, *Calliphora vomitoria* (Linnaeus, 1758), *Chrysomya albiceps* (Wiedemann, 1819) and *Chrysomya megacephala* (Fabricius, 1794). *Chrysomya albiceps* was the most abundant species (61.26%), followed by *L. sericata* (29.37%) and *C. vicina* (8.95%). *Chrysomya megacephala* and *C. vomitoria* were sampled in low numbers (**Table 1**). There was a difference between the number of adults reared under controlled and outdoor conditions. *Lucilia sericata* and *C. vicina* were more abundant in controlled conditions than outdoors, unlike *Ch. albiceps* which was more abundant in outdoor conditions. Reduction of the number of individuals of *L. sericata* and *C. vicina* in natural conditions could be related to predation by mature larvae of *Ch. albiceps*, as could be observed at the end of the decomposition process.

Fig. 1A illustrates relative daily abundance of larvae of the most abundant blowflies, during the first 15 days of decomposition. The first larvae of *C. vicina*, *Ch. albiceps* and *L. sericata* were collected on the third day, however, peaks of abundances of these species did not overlap. Throughout the first days, larvae of *L. sericata* and *C. vicina* were more abundant than *Ch. albiceps*. From the eleventh day, *L. sericata* and *C. vicina* began to decrease at the same time as the number of *Ch. albiceps* increased.

For all species collected, the first sites colonized in the pig were the natural openings of the head (eyes, nostrils, mouth and ears). Nevertheless, *L. sericata*, *C. vicina* y *Ch. albiceps* appeared on the third day, *C. vomitoria* on the eighth and *Ch. megacephala* on the tenth day (**Table 2**). Then, the anus was colonized on the fifth day only by *L. sericata* and *C. vicina*, and on the twelfth day by *Ch. albiceps*. During the last two days of larval collection (fourteenth and fifteenth days), the carcass was almost completely colonized just by *Ch. albiceps*, which was concentrated in the head and posterior region, while mature larvae of *L. sericata* and *C. vicina* were displaced to the abdomen.

Winter

In this season, the primary species were *Calliphora vicina* (96.3%) and *Calliphora vomitoria* (3.7%) (**Table 1**). In both controlled and outdoor conditions, there was a higher number of *C. vicina* than *C. vomitoria*.

The first larvae of *C. vicina* were collected on the fourth day, and this was the only species recorded until the ninth day when larvae of *C. vomitoria* were collected (**Fig. 1B**, **Table 2**). During the first days, *C. vicina* was found in the

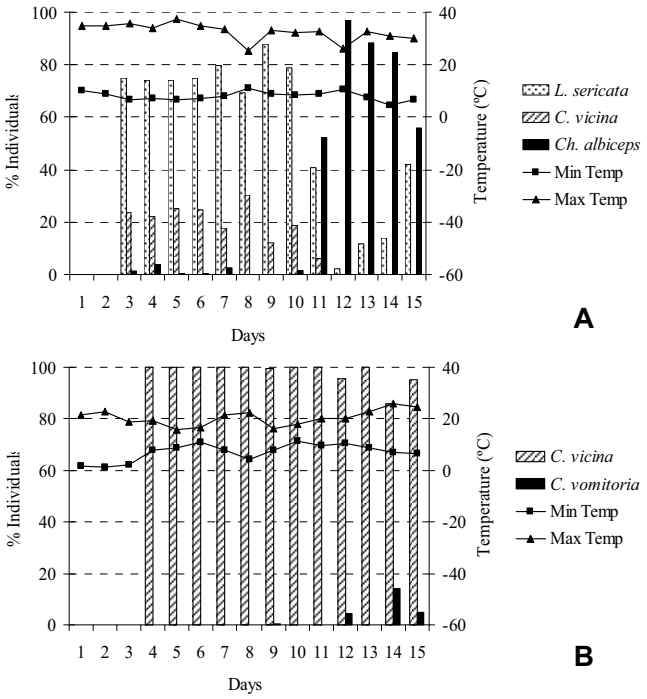

Fig. 1 Relative abundance daily of main blowfly larvae collected from a pig in autumn (A) and winter (B).

Table 2 Occurrence of blowfly larvae on a pig carcass exposed for 10 days.

Species	Days									
	1	2	3	4	5	6	7	8	9	10
Autumn										
C. vicina	-	-	x	x	x	x	x	x	x	x
Ch. albiceps	-	-	x	x	x	x	x	x	x	x
L. sericata	-	-	x	x	x	x	x	x	x	x
C. vomitoria	-	-	-	-	-	-	-	x	x	x
Ch. megacephala	-	-	-	-	-	-	-	-	-	x
Winter										
C. vicina	-	-	-	x	x	x	x	x	x	x
C. vomitoria	-	-	-	-	-	-	-	-	x	x

x = presence; - = absence.

mouth, then, on the seventh day larvae were also localized in the anus. First of all, *C. vomitoria* larvae were collected from the mouth and from the twelfth day, both *C. vicina* and *C. vomitoria* were found colonizing the mouth and anus.

DISCUSSION

This study attempted to identify the main carrion-breeding species susceptible to use for forensic purposes in the Iberian Peninsula, with special interest in the early stages of decomposition. The results obtained from the occurrence table and daily activity could be used as a reference to compare taxa collected from human corpses found in similar environmental conditions. However, to obtain more conclusive results, further studies on colonization with a larger

number of replicate pigs, including more seasons of the year, would be desirable.

The developmental time of immature specimens and the composition of species breeding in a corpse reflect the time period over which the body has been exposed to insect activity. In this respect and taking into consideration the order of appearance of species that developed in the pigs, we could observe which species were present, and therefore, were indicators for a particular period of time. In autumn we were able to separate two groups of species. The presence of *L. sericata*, *C. vicina* and *Ch. albiceps*, would indicate a corpse with a few days of exposition but the occurrence of *C. vomitoria* and *Ch. megacephala* would suggest a time of death of at least eight days. In winter, the exclusive presence of *C. vicina* provides a wide range of time of nine days, but if *C. vomitoria* also appears, this means that the body has probably been exposed for more than nine days.

The species collected in this study did not differ from blowflies found in other experiments from the Iberian Peninsula, especially those from the arid zone of eastern Spain (Arnaldos *et al.* 2001; Martínez-Sánchez *et al.* 2001; Martínez-Sánchez 2003; Arnaldos *et al.* 2004). However, it is important to mention that this study constitutes the first record of *Ch. megacephala* breeding in natural conditions in Europe. Although adults have been reported recently in Spain, Portugal and Malta (Martínez-Sánchez *et al.* 2001; Ebejer 2007; Moneo and Saloña 2009; Prado e Castro and García 2009), there are no previous data on its larval development. This observation corroborates previous data about the permanent presence of this species in Southwestern Europe (Martínez-Sánchez *et al.* 2007). Therefore, the relevance of *Ch. megacephala* and its use to estimate PMI in Mediterranean regions is confirmed.

We observed very aggressive behaviour of *Ch. albiceps*. As reported by Faria *et al.* (1999), Faria and Godoy (2001) and Grassberger *et al.* (2003), larvae of *Ch. albiceps* prey upon other blowfly larvae when carcass remains are not sufficient for development of all larvae. Predation of *Ch. albiceps* could affect a forensic examination because this species is able to displace and eliminate larvae that were previously present. Therefore, an interval calculated based on the *Ch. albiceps* might not correspond to reality and be shorter because it does not consider the development time of earlier species. It will be necessary to study interactions between coexisting species, such as competition, because these may play an important role in successional dynamics and early decomposition processes.

Additional studies on colonization, succession and interactions between carrion-breeding species will help establish baseline forensic entomological information for the Iberian Peninsula, and allow the application of forensic entomology techniques in the Mediterranean region.

ACKNOWLEDGEMENTS

This work is part of a PhD project supported by the Programme Alßan (European Union Programme of High Level Scholarships for Latin America, scholarship No. E06D101359VE) and has been partially supported by the University of Alicante (Project GRE09-27).

REFERENCES

Arnaldos MI, Romera E, García MD, Luna A (2001) An initial study on the succession of sarcosaprophagous Diptera (Insecta) on carrion in the southeastern Iberian peninsula. *International Journal of Legal Medicine* **114**, 156-162

Arnaldos MI, Romera E, Presa JJ, Luna A, García MD (2004) Studies on seasonal arthropod succession in the southeastern Iberian Peninsula. *International Journal of Legal Medicine* **118**, 197-205

Byrd JH, Castner JL (2010) Insects of forensic importance. In: Byrd JH, Castner JL (Eds) *Forensic Entomology. The Utility of Arthropods in Legal Investigations*, CRC Press, Boca Ratón, pp 39-126

Castillo M (2002) Estudio de la entomofauna asociada a cadáveres en el Alto Aragón (España). *Monografías de la Sociedad Entomológica Aragonesa* **6**, 1-94

Ebejer MJ (2007) The ocurrence of *Chrysomya megacephala* (Fabricius) (Diptera: Brachycera) in Malta and records of other Calliphoridae from Maltese Island. *Entomologists Monthly Magazine* **143**, 165-170

Erzinçlioğlu Z (1996) *Blowflies*, Richmond Publishing Co., Cambridge, 72 pp

Faria LDB, Godoy WAC (2001) Prey choice by facultative predator larvae of *Chrysomya albiceps* (Diptera: Calliphoridae). *Memórias do Instituto Oswaldo Cruz* **96**, 875-878

Faria LDB, Orsi L, Trinca LA, Godoy WAC (1999) Larval predation by *Chrysomya albiceps* on *Cochliomyia macellaria*, *Chrysomya megacephala* and *Chrysomya putoria*. *Entomologia Experimentalis et Applicata* **90**, 149-155

González-Mora D (1989) Los Calliphoridae de España, II: Calliphorini (Diptera). *Eos-Revista Española de Entomología* **65**, 39-59

González-Mora D, Peris SV (1988) Los Calliphoridae de España: 1: Rhiniinae y Chrysomyinae (Diptera). *Eos-Revista Española de Entomología* **64**, 91-139

Grassberger M, Friedrich E, Reiter C (2003) The blowfly *Chrysomya albiceps* (Wiedemann) (Diptera: Calliphoridae) as a new forensic indicator in Central Europe. *International Journal of Legal Medicine* **117**, 75-81

Martínez-Sánchez A (2003) Biología de la comunidad de Dípteros necrófilos en ecosistemas del sureste de la península Ibérica. PhD thesis, Universidad de Alicante, 577 pp

Martínez-Sánchez A, Gobbi P, Velásquez Y, Rojo S (2007) Biology of *Chrysomya megacephala* (Fabricius, 1794) in Europe, new data and implications for forensic entomology research (Diptera: Calliphoridae). In: *5th Meeting of the European Association for Forensic Entomology*, 2-5 May, Brussels, Belgium, p 42 (Abstract)

Martínez-Sánchez A, Marcos-García MA, Rojo S (2001) First collection of *Chrysomya megacephala* (Fabr.) in Europe (Diptera: Calliphoridae). *Pan-Pacific Entomologist* **77**, 240-243

Martínez-Sánchez A, Marcos-García MA, Rojo S (2005) Biodiversidad de la comunidad de dípteros sarcosaprófagos en ambientes insulares del sudeste Ibérico (Diptera, Calliphoridae, Muscidae, Sarcophagidae). *Nouvelle Revue of Entomologie* **22**, 251-265

Martínez-Sánchez A, Rognes K, Báez M (2002) Calliphoridae. In: Carles-Tolrá Hjorth-Andersen M (Ed) *Catálogo de los Diptera de España, Portugal y Andorra (Insecta)*, Sociedad Entomológica Aragonesa, pp 204-205

Martínez-Sánchez A, Rojo S, Marcos-García MA (2000a) Annual and spatial activity of dung flies and carrion in a Mediterranean holm-oak pasture ecosystem. *Medical and Veterinary Entomology* **14**, 56-63

Martínez-Sánchez A, Rojo S, Marcos-García MA (2000b) Sarcofágidos necrófagos y coprófagos asociados a un agroecosistema de dehesa (Diptera: Sarcophagidae). *Boletín de la Asociación Española de Entomología* **23**, 148-150

Martínez-Sánchez A, Rojo S, Rognes K, Marcos-García MA (1998) Califóridos con interés faunístico en agroecosistemas de dehesa y catálogo de las especies ibéricas de Polleniinae (Diptera: Calliphoridae). *Boletín de la Asociación Española de Entomología* **22**, 171-183

Moneo J, Saloña M (2009) Primera cita de *Chrysomya megacephala* (Fabricius) (Diptera: Calliphoridae) en la comunidad del País Vasco. *Boletín de la Sociedad Entomológica Aragonesa* **44**, 528-530

Peris SV, González-Mora D (1991) Los Calliphoridae de España, III. Luciliini (Diptera). *Boletin de la Real Sociedad Española de Historia Natural (Sección Biologia)* **87**, 187-207

Prado e Castro C, García MD (2009) First record of *Chrysomya megacephala* (Fabricius, 1794) (Diptera, Calliphoridae) from Portugal. *Graellsia* **65**, 75-76

Prado e Castro C, Serrano A, Arnaldos MI, García MD (2008) Calliphoridae succession patterns from two locations in Portugal. In: *Proceedings of the 6th Meeting of the European Association for Forensic Entomology*, 20-24 May, Kolymbari, Crete, p 43 (Abstract)

Rognes K (1994) First record of the sheep green bottle fly *Lucilia cuprina* (Wiedemann) from Europe (Diptera: Calliphoridae). *Eos-Revista Española de Entomología* **69**, 41-44

Rognes K, Paterson HEH (2005) *Chrysomya chloropyga* (Wiedemann, 1818) and *C. putoria* (Wiedemann, 1830) (Diptera: Calliphoridae) are two different species. *African Entomology* **13**, 49-70

Romera E, Arnaldos MI, García MD, González-Mora D (2003) Los Sarcophagidae (Insecta, Diptera) de un ecosistema cadavérico en el sureste de la Península Ibérica. *Anales de Biología* **25**, 49-63