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Blowflies (Diptera: Calliphoridae) As Forensic Indicators in Egypt with Special Reference to the Development Data of *Lucilia Cuprina* (Wiedemann)

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Abstract
The attraction of Calliphoridae flies, as initial colonizers, to human corpses within a short period following exposure was well studied. However, access to helpful data regarding blowflies' succession patterns is lacking in Egypt. Calliphoridae provides an important source of evidence for forensic/ criminal investigations. However, climate change is altering the distribution of invertebrate pests, including forensically important insects. A modern and continuous record of succession patterns of insects in Egypt will give crucial information and assist forensic investigators. Moreover, examining the link between the geographic distribution of insects and the location-specific climatic conditions will aid in assuming the future insects' distribution scenarios, which are needed in assessing the post-mortem interval. This review recorded several succession models of blowflies on vertebrate carrion in Egypt. Also, it focused on the impact of the regional environmental variations on the life cycle of *Lucilia cuprina*, one among the primary flies to occupy a cadaver upon its death.
Keywords: Calliphoridae; Development data; Forensic entomology; Medicocriminal entomology; Succession patterns; Thermal requirements.

1. Introduction
Forensic entomology is a well-researched approach dealing with the involvement of necrophagous arthropods in investigations of death. Arthropods could be used in both criminal and noncriminal investigations. These investigations are principally necessary to estimate the minimum post-mortem interval (PMI), which is the minimum time between death and the discovery of a corpse. Minimum PMI could be estimated either by the analysis of arthropods' colonization pattern of the carrion or by tracing the development of the immature flies on the carrion. Each method will depend on several factors like season, corpse location and climate [1]. Forensic entomology was proven to be a more precise tool in estimating PMI for intervals longer than 72 h [2]. In addition, forensic entomology can help to identify the sites of trauma and post-mortem artifacts on the body as to discover gunshot residues in decomposing tissues; to determine the period of neglect of living person with infested wound and to associate the victim and suspect to each other and to the scene [3-7]. In entomotoxicology, which points to the presence of medications or poisons in decomposing tissues of cadavers, it was found that these substances might change the rate of insect aggression and their growth rates [8-14]. However, Abd El-Bar, *et al.* [15], observed no differences in the insect's community composition between the control carcasses and the intoxicated groups by zinc phosphate. Interestingly, insects were proven to be useful as an alternative toxicological indicator in cases of severely decomposed corpses or missing ones [16, 17]. On the other hand, Foraging activity of dipteran flies from crime scenes and laboratory experiments showed counterproductive role in criminal investigations as they could disrupt crime scene and flies regurgitation/defecation spots/spatters couldn't be easily differentiated from human body fluids [7, 18-20].

The linkage between climate change and insect distribution are still lacking in Egypt. This change is a serious issue that has an impact on the parasite's distribution globally [21]. The insects' pattern of succession could vary, from one place to another, according to the zoogeographic region, type of victim death, and climatic changes that influence the rate of insect development, survival, and reproduction [22, 23]. This review aimed to describe the recent succession patterns of blowflies in Egypt, and the importance of their development data in forensic analyses. It, also, sheds light on *Lucilia cuprina* development data from different countries including Egypt, to emphasis the impact of seasonal and geographical conditions on the accuracy of PMI determination.

2. Main Text
2.1. The Succession of Blowflies to Determining the PMI
Faunal succession is an expecting sequence of dead corpse's colonization, by arthropods, soon after death [24]. The information of the time passed until the first appearance of particular arthropod species on a cadaver could be

used to assess PMI [25].insects are the most constant and diverse arthropod group that dominate planet earth and present in all situations, except in submerging situations [11, 26]. There are several insects, which can be found around decomposing carrion, including necrophagous species feeding on the carrion; predators and parasites feeding on the necrophagous species; omnivorous species feeding on the carrion and other arthropods [27]. Using a succession pattern means that, an investigator will have to deal with numerous complicating factors [28]. Those factors will include the temperature inside the corpse, which is often much higher than the air temperature, and it varies according to the state of decomposition and the location of insects within the corpse [29, 30]. The size of the corpse should be taken into consideration, as the temperature in small cadavers would not reach the high levels found in large cadavers. Moreover, post-feeding larvae may crawl distant away from the corpse and the development time of those stages would, therefore, be affected by air temperature [31]. Besides, the length of the pre-infestation period, as well as the rate of development, may be affected by drastic and sudden environmental conditions in the perimeter of the corpse [32-34].

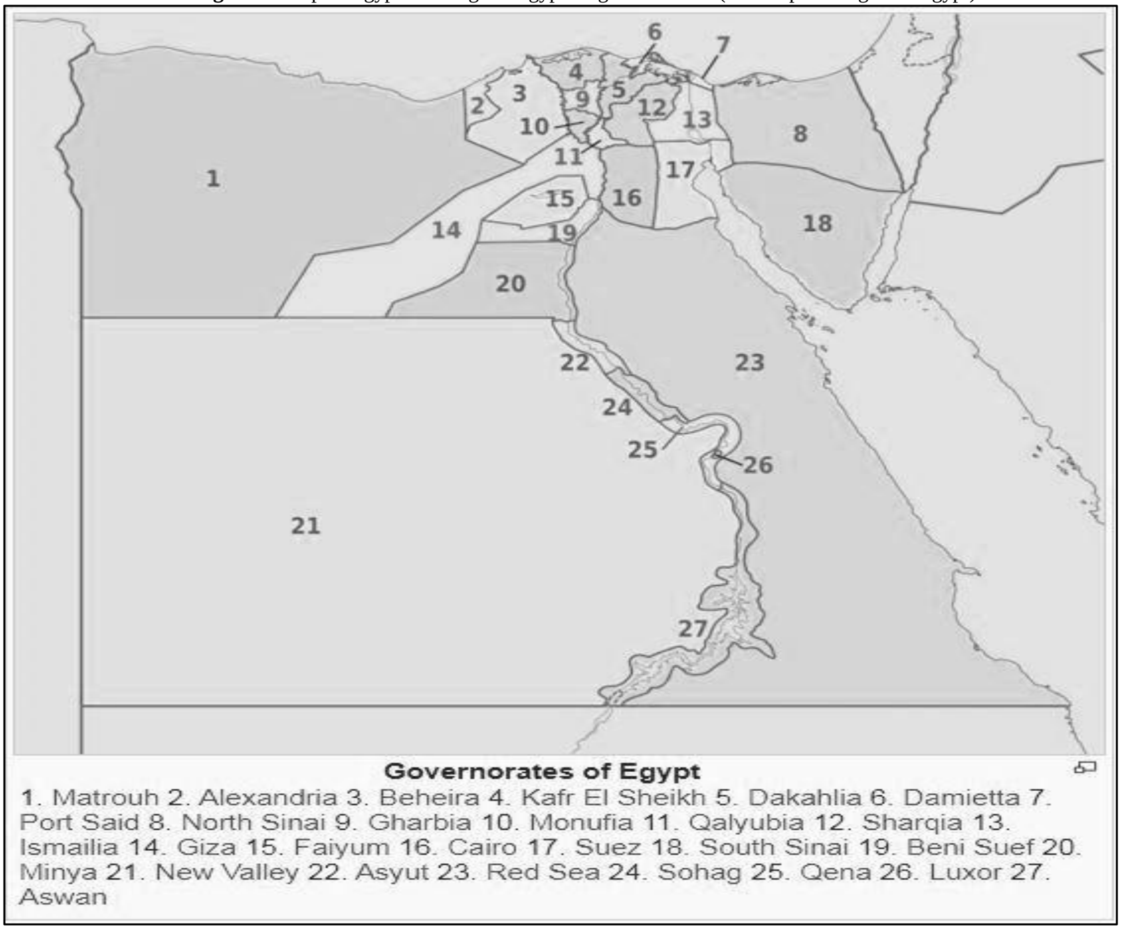
Both Diptera and Coleoptera include the most important families which are supporting the decomposition of cadavers [35]. The utmost depletion of terrestrial carrion is due to maggots of blowflies and flesh flies, in addition to several other flies that exist as the decomposition progresses [36, 37]. The identification of the initial colonizers of cadavers as well as their habitat is crucial, as it provides important information to estimate the PMI and whether the body has been moved from its original location [38-40]. Blowflies are attracted to human corpses within a few minutes [33, 41-43] or even seconds [44] following carrion disclosure, as they have a strong physiological drive to reach their food resource and oviposition site. The attraction of adult calliphorids to a corpse may be due to putrative sulfur-based compounds and ammonium-rich compounds emitted from freshly decomposing tissues [45] or due to the pheromonal signals which draw the attention of gravid females to the carrion [46, 47]. Gravid females deposit eggs in mucosal body openings, and/ or in body lesions and wounds [48]. Blowflies are encountered more frequently in the first few weeks of discovering body corpses; therefore, it could be used to detect minimum PMI more accurately than other carrion insects [33, 38]. *Lucilia cuprina* is one of the first flies to colonize a corpse upon its death [49, 50]. Although this fly has been found associated with animal carcasses and human corpses in various regions [15, 48, 51-54], it was known as specific colonizer to dryer climate in India [55]. The blowfly maggots elevate temperature in carcasses, and this may have several evolutionary advantages including increased efficiency of food processing, gaining a competitive advantage over potential arrivals, reducing the predation level against them by accelerating development [56, 57], and mitigating the effect of climatic conditions [58]. Various species of blowflies are characterized by specific developmental biology, ecology, and behavior [59]. A carrion-related blowflies' database is important for each specific habitat and time, as the involved species varied throughout the year [60].

The insects' composition and succession patterns on carrion have been studied in a few cases from several Egyptian habitats (Table 1, Fig. 1). Calliphorids were the initial colonizers and the dominant species in the major models [8, 28, 61]. By contrast, few authors [34, 62] recorded members of Sarcophagidae and Muscidae as the initial and dominant colonizers on carcasses in Ismailia and Qena (Table 1, Fig. 1). Aly, *et al.* [34], recorded the presence of *Lucilia sericata* and *Chrysomya albiceps* among the dipteran species that constituted the primary factor in the decomposition process of carcasses. The high temperature and relative humidity in Egypt have accelerated the decomposition process leading to a rapid depletion of the food resource and reduction of arthropod colonization time [34]. An earlier study reported *L. sericata*, *Lucilia illustris*, *Calliphora vicina* and *Pollenia* sp. from the natural ecosystem in the Egyptian western desert [63]. The authors recorded the presence of *L. illustris* for the first time in the western desert and the Mediterranean region. They found also that, the members of Calliphoridae were predominant (56%) in traps baited with fish where *Lucilia* sp. disappeared or were present in very low numbers during winter months. Rabbit carcasses in Alexandria attracted *C. albiceps* and *L. sericata* during the fall and spring of 1988-1989 [28]. The later noticed that *L. cuprina* was extremely scarce and did not breed on carrion. Bait traps at different localizations in Suez province, *Chrysomya megacephala* was the most dominant among different sacrosaprophagous species [64]. The authors caught several other blowflies like *L. cuprina*, *C. albiceps*, *L. sericata and C. vicina.* In El-Qalyubiya, necrophagous insects showed species' high dispersal abilities, shortly following death, for *C. albiceps* which constituted 76.6% [8]. The previous study recorded *Chrysomya marginalis* as a carrion breeding species. Another study, in the same area, was conducted on 11 domestic guinea pig carcasses from April 2009 to March 2010 [65]. The authors recorded the presence of C. *albiceps* adults at the first four days of the experiment (Fresh and Bloating stages), then the fly visited the carcasses occasionally at day 36 (Dry stage). In Cairo, a succession model on decayed rabbits and guinea pigs revealed that calliphorid flies were the first insect family that colonized the different carcasses and lasted till the end of the active decay stage [15]. The authors recorded the presence of *C. albiceps*, *C. megacephala*, *Chrysomya rufifacies*, *L. sericata* and *L.cuprina* during winter and summer seasons of 2012. Also, they documented the early succession of *C. albiceps*, which was recorded in other global regions. Recently, a study identifying *Chrysomya* species in Cairo has proved that *C. albiceps* was the most abundant species followed by *C. megacephala*, however, only three flies of *C. marginalis* were collected among 76 specimens during June-September 2014 [66]. In a comparative study in Qena governorate, the authors collected twelve species of Diptera and three species of Coleoptera and Hymenoptera from rabbit carcasses through the four seasons [62]. The dipteran species were the predominant groups on the outdoor and indoor experiments, where *Sarcophaga carnaria* is the primary and the most frequent colonizer in various habitats, and both *C. albiceps* and *L. cuprina* could be used as PMI indicators [62]. Although *C. albiceps* is not recommended being used as a biogeographic indicator due to its wide distribution over Egypt, it still one of the most reliable flies in the estimation of PMI along with *L. cuprina* [28, 61, 67, 68].

Table-1. Location of the previous studies of succession patterns of insects on carrion in Egypt

The study	Region	Location in Figure 1	Geographic location	Latitude (N)	Longitude (E)
Hegazi, *et al.* [63]	Western desert	1	Matrouh Governorate, north-western Egypt.	29.5696°	26.4194°
Tantawi, *et al.* [28]	Alexandria	2	East of Matrouh, north-western Egypt.	31.2001°	29.9187°
Gabre and Abou [64]	Suez province	17	North-eastern Egypt, near the southern terminus of the Suez Canal.	29.9668°	32.5498°
Aly, *et al.* [34]	Ismailia	13	North-eastern Egypt, located on the west bank of the Suez Canal.	30.5965°	32.2715°
Abd El-Bar and Sawaby [8] Ibrahim, *et al.* [65]	El-Qalyubiya	11	North of Cairo in the Nile Delta region.	30.3292°	31.2168°
Zeariya, *et al.* [69] Abd El-Bar, *et al.* [15] Elleboudy, *et al.* [66] Fouda, *et al.* [13]	Cairo	16	Egypt's capital, south to the Nile Delta and north to upper Egypt.	30.0444°	31.2357°
Galal, *et al.* [61]	Asyut	22	Upper Egypt, 375 KM South of Cairo.	27.1783°	31.1859°
Aly, *et al.* [62]	Qena	25	Upper Egypt, 600 KM South of Cairo	26.209°	32.768°

Figure-1. Map of Egypt showing the Egyptian governorates. (en.wikipedia.org/wiki/Egypt)



2.2. The Role of Blowflies' Development Data in Determining PMI

Forensic entomologists use size and development stages to estimate blowfly age, and consequently, a post-mortem interval [70]. Accurate forensic analyses need species identification, for all insect species found in human and/or at death scenes, as the lower development threshold and thermal summation constants are distinct between different species of the same genus [71]. Most development data have been obtained for blowflies because they are pivotal and typically the first to find a body [38, 72-74]. The models that describe insect development are temperature driven, as temperature affects insect phenology and distribution [75-81]. Therefore, the previous weather forecasting should be noted upon taking larval samples from decomposed corpses to estimate the correct time of death [82]. Also, the larval growth rate of blowfly species was found to be affected significantly by the tissue types and species tissue source [19, 83, 84] Due to genetic diversity and variation of environmental factors, some variations in the development time for geographically distinct populations within the same blowfly species were documented around the world [85-89] including Egypt [68, 90-92]. Large aggregations of maggot masses in a corpse develop heat due to their frenetic activity and fast metabolism, thus raising the micro-environmental temperature [29]. Regardless of the season, maggot-mass temperatures ranged between 35-45 °C and 20 °C higher than ambient low temperature and ambient high temperatures respectively [93]. Some species may regulate internal aggregation temperatures (particularly upper limits) because not all necrophagous flies tolerate high temperature in the maggot masses equally [94].

The variation of development times between different populations of *L. cuprina*, as an important and reliable biological indicator in the estimation of PMI, was proved to be a specific characterization of surrounding conditions (Table 2). For example, one life cycle (egg deposition till adult emergence) decreased by 389.08 h (16.21 days) in the summer season compared to winter in Giza, Egypt [90]. The later author recorded acceleration in life cycle of *L. cuprina* by 43.36 h (1.81 days) at controlled temperature 32 °C compared to summer laboratory experiment (mean temperature 31 °C±3), however, a delay by 170.94 h (7.12 days) was detected under the controlled temperature 17 °C compared to winter laboratory experiment (mean temperature 20 °C±3). Generally, the development rate of *L. cuprina* was faster at warmer weather and high humidity [49, 90]. In several studies, *L. cuprina* completed its life cycle well around 30 ºC [90, 95], larval length peaked at 27 °C [96] and larvae survived best at 24 ºC [95, 96]. At lower temperature, eggs failed to hatch at 9 ºC [50], adults failed to lay eggs at 17 ºC in complete darkness [90] and larval development was slower and survival was compromised at a temperature lower than 24 ºC [90, 96]. O'Flynn [50] found that *L. cuprina* completed its larval duration but failed to pupate at a constant temperature 40 ºC, however, Bansode, *et al.* [49] determined a complete life cycle at the same constant temperature. Both authors neglected to mention the humidity ranges and/ or the photoperiodism. Therefore, it was obvious that the development cycle of the same species could vary at the same constant temperature if the other factors were different (Table 2), which might add difficulties to the work of the forensic investigators to use insect's age and length as reliable indicators to estimate PMI. The methods used in forensic cases, for maggot age determination, generally involve a comparison of sample maggots to maggots of known ages which were reared under constant condition [97-101]. However, under field conditions temperature was rarely constant and it has been shown that temperature fluctuations differently affected the development of maggots and the fly's strategy in nature [58, 102]. Day and Wallman [84] favored constant temperature to develop reliable data on *Calliphora augur* and *L. cuprina* because fluctuating regimes would be location specific and may, therefore, be of limited application. However, the development data of blowflies obtained from laboratory readings could be applied directly to similar indoor situations to field cases in which the temperature is a little fluctuated and/ or to the post-feeding larvae and pupae which crawled in the soil out of corpses [93, 103]. Accordingly, upcoming regional researches should emphasize on the field rather than laboratory studies.

Table-2. The development data of *Lucilia cuprina* at constant and laboratory temperatures from several studies around the world, including Egypt

Reference	country	Temperature (°C)	RH (%)	L: D	Incubation period	Larval duration	Pupal duration	Egg-adult development	Preoviposition period	No. of eggs/ female/ one batch
O'Flynn [50]	Australia	15*	-	-	~45 h	18- >40 d	~25 d	~43 d	-	-
		20*	-	-	26 h	7-10 d	13-14 d	21-22 d	-	-
		25*	-	-	~13-19 h	-	-	-	-	-
		28*	-	-	-	4-10 d	7 d	12-13 d	-	-
		34*	-	-	-	3.5-6 d	6 d	10-11 d	-	-
El-Bassiony [90]	Egypt	17*	55 ± 5	CD	32.33 h	486 h	338.64 h	35.71 d	-	NA
		20**	60 ± 5	11:13	24.83 h	297.12 h	364.08 h	28.58 d	230.4 h	82.8
		27*	69 ± 5	CD	13.67 h	150 h	193.92 h	14.90 d	80.4 h	NA
		31**	50 ± 5	14:10	10.83 h	117.12 h	169 h	12.37 d	86.4 h	141.15
		32*	74 ± 5	CD	11.67 h	93.12 h	148.80 h	10.57 d	70.32 h	NA
Kotzé, *et al.* [96]	South Africa	18*	-	-	-	-	~19 d	-	-	-
		21*	-	-	-	-	~9 d	-	-	-
		24*	-	-	-	-	~7.1 d	-	-	-
		27*	-	-	-	-	~7.5 d	-	-	-
		30*	-	-	-	-	~7.7 d	-	-	-
		33*	-	-	-	-	~5 d	-	-	-
Barros-Cordeiro, *et al.* [104]	Brazil	23*	60 ± 10	12:12	-	-	210 h	354 h	-	-
Bansode, *et al.* [49]	India	20*	50-86	-	13 h	217 h	410 h	26.67 d	-	-
		25*	56-72	-	15 h	195 h	336 h	22.75 d	-	-
		30*	60-79	-	16.5 h	144.5 h	193 h	14.75 d	-	-
		35*	58-65	-	17.5 h	144	144 h	12.73 d	-	-
		40*	60-70	-	15.5 h	135.5 h	122 h	11.38 d	-	-
Hasan [105]	Jordan	25**	-	-	18.5 h	101.76 h	180.96 h	12.78 d	24-72 h	58
Ishak, *et al.* [106]	Malaysia	29*	75	10:13	-	184 h	244 h	-	-	-

3. Conclusions

Blowflies are known as the first insects that colonized carcasses and corpses after the death. Regional studies, with the scientifically documented relationship between climatic changes and biological parameters of insects, are desirable to increase the precision of PMI estimation in local medicolegal investigations. Local data could constitute a useful basis for forensic entomology investigations, especially when they include all surrounding conditions in detail. This study provides the forensic entomology field with a calibration of the effect of temperature on the development rate of *L. cuprina* in Giza, Egypt. It is hoped that this review may begin to fill the knowledge gap, as well as to stimulate further studies of succession models and patterns in Egypt.

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