Original Article

Temperature Requirements of Some Common Forensically Important Blow and Flesh Flies (Diptera) under Laboratory Conditions

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Abstract

Background: The aim of his study was to determine development time and thermal requirements of three myiasis flies including *Chrysomya albiceps, Lucilia sericata,* and *Sarcophaga* sp.

Methods: Rate of development (ROD) and accumulated degree day (ADD) of three important forensic flies in Iran, *Chrysomya albiceps, Lucilia sericata,* and *Sarcophaga* sp. by rearing individuals under a single constant temperature (28° C) was calculated using specific formula for four developmental events including egg hatching, larval stages, pupation, and eclosion.

Results: Rates of development decreased step by step as the flies grew from egg to larvae and then to adult stage; however, this rate was bigger for blowflies (*C. albiceps* and *L. sericata*) in comparison with the flesh fly *Sarcophaga* sp. Egg hatching, larval stages, and pupation took about one fourth and half of the time of the total pre-adult development time for all of the three species. In general, the flesh fly *Sarcophaga* sp. required more heat for development than the blowflies. The thermal constants (K) were 130–195, 148–222, and 221–323 degree-days (DD) for egg hatching to adult stages of *C. albiceps*, *L. sericata,* and *Sarcophaga* sp., respectively.

Conclusion: This is the first report on thermal requirement of three forensic flies in Iran. The data of this study provide preliminary information for forensic entomologist to establish PMI in the area of study.

Keywords: Degree Day, Forensic Entomology, Larval development, Myiasis, PMI

Introduction

Determination of postmortem interval (PMI) or the time between death and the discovery of a corpse is the most important application of forensic entomology. Flies belong to the families Calliphoridae (blow flies) and Sarcophagidae (flesh flies) are often the first insects to arrive on a corpse where their larvae feed and breed effectively (Anderson 2001, Dadour et al. 2001, Higley and Haskell 2010). Development rates of these flies are frequently used to estimate PMI in homicide investigations in the first few weeks after death. Since development of immature insects is temperature-dependent, PMI is normally calculated by the accumulated degree day/ hour (ADD/ADH) model (measure of thermal time taken to reach each developmental event, K) which is associated with basal temperature called the lower temperature threshold (T_L) or the developmental zero (Dz) (temperature below which development ceases) values (Nietschke et al. 2007, http://www.ipm.ucdavis.edu/MODELS, Oshaghi et al. 2009). The rate of larval growth depends on its body temperature, which is directly affected by environmental conditions as ambient tem-

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perature and the heat generated by maggot aggregations (Slone and Gruner 2007). In addition, an important detail for PMI determination is that each species has its own temperature dependent growth rate.

Generally life cycle of blow and flesh flies includes four stages of egg, larval, pupal and imago. Three instars can be seen in the larval stage: 1st, 2nd and 3rd instars, where the latter is divided to feeding and post-feeding larvae (Day and Wallman 2008). The flies deposit egg directly on the food substrate to ensure a food supply for the hatching 1st instar larvae. The three instars can be distinguished by the number of respiratory slits at the posterior end of the larvae. The third instar stage lasts longer than the first two ones, and the post-feeding stage is a preparation phase for pupation (Reibe et al. 2010). About one third of the pre-adult development time is spent in the post-feeding larval stage (Greenberg 1991). Therefore, the larvae leave the food source to find a suitable place for pupation, emptying their gut (Arnott and Turner 2008). During pupation stage the imago develops within the pupal case till eclosion and takes about half of the time of the total development (Reibe et al. 2010).

Blow flies such as *Chrysomya albiceps* (Wiedemann) and *Lucilia sericata* (Meigen) (Diptera: Calliphoridae) and flesh flies such as *Sarcophaga* spp. (Diptera: Sarcophagidae) are widely distributed throughout the world and characterized as a facultative ectoparasites responsible for primary or secondary myiasis in humans and livestock (Zumpt 1965, Smith 1986, Hall and Wall 1995, Anderson, 2000, Grassberger et al. 2003). These species are considered as sinantropic species, i.e. it is in close relation with human settlements. They also feed on carrion and human feces, and breeds prolifically in carrion, making them medically, veterinarily, sanitationarily, and forensically important flies (Zumpt 1965, Grassberger et al. 2003). They are recognized as the first wave of the faunal succession on human cadavers and are the primary and most accurate forensic indicators of time of death. *Lucilia sericata* also has a significant role in human medicine because its larvae are used for healing chronic injuries that do not respond to conventional treatments, such as ulcers containing gangrenous or necrotic tissue (Church and Courtenay 2002, Cartier and Combemale 2008, Gupta 2008).

The biology of blow flies and flesh flies have been studied previously on many aspects such as age, mortality rates and reproductive output, thermal requirements, diet, minimum and maximum threshold temperatures, and development duration under field or laboratory conditions (Kamal 1958, Denlinger 1972, Aspoas 1991, Greenberg 1991, Smith and Wall 1998, Hayes et al. 1999, Anderson, 2000, Grassberger and Reiter 2001, Al-Misned 2003, Pitts and Wal 2004, Farkas et al. 2005). However, there has been less research on life table parameters carried out with samples of *C. albiceps*, *L. sericata*, and *Sarcophaga* sp. from an oriental country like Iran. Due to the recent increase of forensic entomology utilized in Iran, and the importance of an understanding of the rate of development in relation to temperature, detailed development data are needed to allow more precise postmortem interval (PMI) estimates.

In this paper, we present development rate of these three important forensic flies in Iran (Fig. 1), at a single constant temperature and calculate the ADD/K required for four developmental events including egg hatching, larval stages, and pupation, and egg-eclosion. We compared the K of our findings to those of published data to explore the disparities between the studies. These information can also support the mass rearing of them under laboratory conditions to be used in maggot-therapy (in case of *L. sericata*) and can also contribute to the monitoring and providing control strategies of these dipteran flies.

Materials and Methods

Sample collection

Adult flies were collected using plastic bottle fly traps and entomological nets at the Laleh Park in center of Tehran as well as the livestock shopping center and close vicinity of slaughter houses in the east and south of Tehran, Iran. The traps were made by cutting the top of a plastic water bottle, placing some sands (3 cm) into the bottom of the bottle, putting some raw sheep/chicken liver (100 g) on top of the sand, and then inverting the top of the bottle into the bottom, and tape the two halves together. Larval collection from natural infestations of sheep and cattle were performed, but it was unsuccessful because of insecticide application on the animals.

The captured flies in entomological nets immediately transferred into glass jars. The jars or baits harboring flies were then transported in a polystyrene icebox to the laboratory of Medical Entomology, School of Public Health, Tehran University of Medical Sciences. Insect collections were carried out from late spring to early summer 2010, beginning in mornings and continued until a sufficient number of specimens for the colonization process had been captured.

Maintenance of flies in the laboratory

Adult were transferred individually into a bottle trap including sand and meat to lay eggs at 28° C±1, 40%±5 relative humidity and 12 h photoperiodicity, protected with an external net curtain to avoid the entry of other insect species. After laying eggs, the dead specimens were identified morphologically by using the taxonomic keys of James 1947, Zumpt 1965, McAlpine 1981, and Whitworth 2006.

Determination of Development Rate and ADD (K)

Groups of 30 plastic bottles were placed in incubators set at the specified constant temperature (28° C±1). The bottles were ventilated daily, and moved within the incubators to minimize the effect of any systematic temperature gradients. Life cycle duration of *L. sericata, C. albiceps,* and *Sacophaga* sp. were determined over two consecutive generations by recording the average time in days, for different stages of each species.

Recording the time required for egg hatching, larval stage developments, and pupation, and total time for egg-eclosion was performed every three hours intervals for eggs and every five-six hours intervals for larvae and pupa. On each recording occasion, at least five bottles checked on a light microscope. The rates of development (ROD) were measured for each life stage by inversion of developmental duration (ROD=1/Day). Baseline temperature or lower threshold temperature (Dz) for each developmental stage of the species was obtained from previous studies (Table 1). Because the Dz values for each species varied between studies, in this study we used an average value wherever more than one data set was available. Hence, the average estimates of lower developmental thresholds of *C. albiceps* prepared by Marchenko (1988), Queiroz (1996), Grassberger et al. (2003), and Richards et al. (2008) was accounted for egg (9.72° C), larvae (12.93° C), pupae (12.55° C), and egg to adult (11.73 ^{o} C). The estimates of the basal temperature of *L. sericata* for whole life stage (egg-adult) was accounted 8° C (Reibe et al. 2010), 8.2° C, 11.3° C (Woodburn et al. 1978), and 9° C (Marchenko 2001, Niederegger et al. 2010). Therefore, in this study the average 9.5° C was used for *L. sericata*. For *Sarcophaga* sp. we used the only information available for a closely related flesh fly, *Sarcophaga dux* Thomson (Al-Misned 2003). Estimates of the lower developmental threshold temperatures for this species were 5.9, 12.9 and 11.0 ^{o}C for larvae, pupae and total developmental time, respectively.

Based on the Dz and duration of the development, the ADD/K for each developmental stage of the dipteran flies was calculated using the formula ADD or K=D (Tm- Dz) where K=degree days (^{o}C), D= developmental duration (days), Tm=the ambient (experimental) temperature (^{o}C), and Dz= base development threshold (^{o}C) (Higley and Haskell 2010).

Results

Generally, the rates of development changed in a decreasing order respectively in eggs, larvae, and pupae of the three species at the laboratory condition (28° C and 40% RH). This value was 0.33–0.50, 0.33–0.50, and 0.16–0.25 for eggs, larvae, and pupae of blowflies accordingly. Development rates for *Sarcophaga* sp. were lower than the blowflies. These rates for *Sarcophaga* sp. were 0.33–0.50, 0.16–0.20, and 0.10–0.16 for eggs, larvae, and pupae respectively. Life cycle durations of the dipteran flies was determined over two consecutive generations, and recording the average time in days, for different stages in the three species (Table I). The development duration of eggs of both blowflies (*L. sericata* and C. *albiceps*) and flesh fly (*Sarcophaga* sp.) was similar but the development duration of larval and pupal stages in the blowflies were shorter than the flesh fly. Life cycle span from egg to eclosion in blowflies ranged from eight to twelve days whereas it was ten to sixteen days in the flesh fly. Egg hatching and larval stages prolonged each about one fourth of the total pre-imago time whereas duration of pupation took almost half of the time of the total pre-adult development time for all of the three species.

In general, the flesh fly *Sarcophaga* sp. required more heat for development than the blowflies. The thermal constants (K) or accumulation degree-days required for egg hatching was 37–56, 37–55, and 44–66 degree-days (DD) for *L. sericata*, *C. albiceps*, and flesh fly *Sarcophaga* sp. respectively. At the larval stage, *C. albiceps* with 30–45 DD required less heat than *L. sericata* with 37–56 and *Sarcophaga* sp. with 44–66 DD. For development of pupa, again *C. albiceps* with 62–93 DD required less heat than *L. sericata* with 74–111 DD and *Sarcophaga* sp. with 91–151 DD. Although, total heat requirement of *C. albiceps* to develop from egg to adult (130–185) was less than the requirement of *L. sericata* (148–222 DD), but total developmental time from egg to adult for both species was similar (8–12 days). It was 13–19 days and 221–323 DD for the the flesh fly.

Table 1. Development duration and thermal requirements (K/ADD) for four developmental events for *L. sericata*, *C. albiceps* and *Sarcophaga* sp.

Life stage	Development Duration (Day)			ADD/K (DD=°C)		
	L. sericata	*C. albiceps*	*Sarcophaga* sp.	*L. sericata*	*C. albiceps*	*Sarcophaga* sp.
Egg- Hatching	2–3	2–3	2–3	37–56	37–55	44– 66
Larvae (L1-L3)	2–3	2–3	5–6	37–56	30–45	44– 66
Pupae	4–6	4–6	6–10	74–111	62–93	91–151
Egg-Adult	8–12	8–12	13–19	148–222	130–195	221–323

Fig. 1. Photographs of *Chrysomia albiceps*, *Lucilia sericata*, and *Sarchophaga* sp. from left to right respectively

Discussion

Results of this research showed highly differences for development rate of accumulated degree days required for blow flies (*C. albiceps* and *L. sericata*) in comparison with flesh fly *Sarcophaga* sp. In addition, rate of developments in various life stages was different and reduced gradually throughout the life from eggs to adult stage in all of the three species. The speed of development in the flesh fly was slower than blowflies. This finding is very important in forensic entomology and PMI where *Sarcophaga* sp. presents. Total developmental time of *Sarcophaga* sp. from first -instar larvae to adult emergence reared at 28° C was 13-19 days which is similar to the total development time (16.4 day) of *Sarcophaga dux* reared at the same temperatures (28° C) (Al-Misned 2003). Total developmental time of *C. albiceps* from egg to adult was 8-12 days when reared at 28° C, which is similar to those (9.5–10.5 days), reported by Marchenko (2001), Al-Misned et al. (2003), and Grassberger et al. (2003) reared at temperatures 30° C.

The duration of the life cycle described in this research for *L. sericata*, from egg to adult was 8–12 days which is more or less shorter in comparison to data provided by Rueda et al. (2010) (14 days), Anderson, (2000) (14 days at 27° C), Usaquén and Camacho (2004) (26 days under natural environmental conditions), Nuorteva, (1977) (23–28 days under field conditions), and Anderson, (2000) (32 days at 16^{o} C and 20 days at 21°C), and somehow similar to the information by Kamal (1958) (12–15 days at 22° C and 50% relative humidity). This disparity between our results and other studies can be mainly explained by two factors; the higher temperature we used and the characterizations of the local populations we studied. It is proved that the development of fly larvae is temperature-dependent, and in higher temperature the rate of development increases and duration of development becomes shortened. Variation has been observed in developmental time for geographically distinct populations (Rechard et al. 2008; Gallagher et al. 2010). The results obtained in this study are the first data for the Iranian forensic flies which are originated from a totally different places than the populations used in other studies. The variation of developmental times between different populations emphasizes on specific characterization of regional developmental times of species.

In the current study we used meat traps which can trap only females. Unfortunately identification of females of Sarcophagidae is very difficult and in most cases is impossible. In this study we tried to prepare some DNA markers (mtDNA COI as well as ITS2 rDNA) for the specimens of *Sarcophaga* sp. found in this study (unpublished data) but due to lack of their counterparts in GenBank we could not identify the specimens and it remained unclassified. Generally only males of this family can be identified, and then only by examination of dissected genitalia (Pape 1996).

In this study we focused only on temperature as a main factor for development of the flies, however, other important factors such as natural or synthetic diet, humidity, and competition between larvae should be accounted for this purpose. The effect of diet on development of flies has been shown in various studies (Kaneshrajah and Turner 2004, Clark et al. 2006, Tarone 2006, Rueda et al. 2010). It was shown that natural diets such as beef liver that are often used in laboratory rearing of flies produce offensive odors and contamination (Sherman and My-Tien Tran 1995). Moreover, it is demonstrated that the presence of toxins in decomposing tissues from natural diets can affect the development rate and generate errors in PMI estimates when necrophagous insects are used for forensic studies in the laboratory (Estrada et al. 2009). Clark et al. (2006) compared the development of the blowfly *L. sericata* fed on lung, liver and heart, from both cows and pigs and observed that larvae grew significantly faster and gave rise to larger adults when reared on pig compared to cow tissue and when reared on lung and heart compared to liver. Also, it is shown that the artificial diet based on powdered milk can cause a lower duration of larval stages in comparison to the animal liver (Tachibana and Numata 2001). Overcrowding of larvae can decrease growth rate resulting in an underestimation of the PMI (Smith and Wall 1997).

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