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Effect of Temperature on the Development of *Lucilia sericata* (Meigen) and *Chrysomya albiceps* (Wiedemann) (Diptera: Calliphoridae) from Alexandria, Egypt.

***A. M. Kheirallah, T. I. Tantawi, A. H. Aly and Z. A. El-Moaty.**
Department of Zoology, Faculty of Science, Alexandria University, Alexandria, Egypt.
**Corresponding Author: email:*

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Abstract

The influence of rearing temperature on the duration of development in two blow flies *Lucilia sericata* and *Chrysomya albiceps* was studied by rearing the insects at four different temperatures (17, 23, 29 and 35ºC). The minimum duration for each development stage, from egg oviposition to adult eclosion, were observed. Data from these studies were used to construct the isomorphen diagram and a temperature dependent model was calculated for each species. Compared to *L. sericata*, *C. albiceps* has a higher threshold of development and a longer duration of larval development. In addition, these results vary from those of other investigators elsewhere, suggesting a different thermal behaviour of these blow flies in various zoogeographic regions.

Key words: *Chrysomya albiceps*, development, *Lucilia sericata*, temperature.

Introduction

Calliphoridae are recognized as the first wave of the faunal succession on human cadavers (Nuorteva, 1977 and Smith, 1986). Therefore, knowledge of the development rate of their larvae is useful to determine the time elapsed since death, the so-called postmortem interval (PMI) (Greenberg, 1991). Insect development is affected by many factors, environmental conditions in particular. Temperature is the most important factor affecting development rate (Myskowiak and Doums, 2002).

The blow fly *Lucilia sericata* is a communicative eusynathrope, which is widespread throughout the major zoogeographical regions, but is not yet cosmopolitan (Greenberg and Povolny, 1971; Smith, 1986; Spradbery, 1991). It is a Holarctic species distributed over the warmer regions of the temperate zone (Hall and Wall, 1995) and now the dominant species in urban and suburban districts of Australia and Africa (Zumpt 1965, Wall *et al.*, 1992a). *L. sericata* is a facultative ectoparasite, which acts as the primary agent of sheep myiasis in Britain and parts of continental

Europe (Smith, 1931; Davies 1934; MacLeod, 1943; Wall *et al*., 1992a; Hall and Wall, 1995; Smith and Wall, 1997), in South Africa (Hepburn, 1943) and Australia (Norris 1959).

Chrysomya albiceps is a hemisynathropic species, which prefers high temperatures and humidity (Greenberg and Povolny, 1971). This tropical and subtropical species is very common and abundant in Africa, southern Europe, tropical and subtropical Arabia, India and recently central and South America (Zumpt, 1965; Guimarães *et al*., 1978 & 1979; Mariluis, 1980; 1981 & 1983; Leite *et al*., 1983; Baumagartner and Greenberg, 1984; Baumagartner, 1988; Spradbry, 1991; Hall and Smith, 1993; Grassberger *et al*., 2003).

Due to the forensic importance of these species, several studies on their development times under different constant temperature regimes were undertaken (for *L. sericata*: Greenberg 1991; Wall *et al*., 1992b; Anderson, 2000; Grassberger and Reiter, 2001 and for *C. albiceps:* Marchenko, 1985; Qeiroz, 1996; Grassberger *et al*., 2003). However, data obtained throughout these studies were not consistent. Our aim is to compare the findings on the development of the two studied species with previous studies and gives some information on the development rate, minimum development threshold and thermal constant of these flies.

Materials and methods

Maintenance of Laboratory Fly Colonies

Larvae and adults of the two species were collected from exposed rabbit carcasses at Moharrem Bey District, Alexandria, Egypt. Larvae and adults were identified to species level according to Tantawi and El-Kady, (1997) and Smith, (1986). The flies were held in an insectary (50 x 50 x 50 cm) at 25 ± 2ºC and supplied with water, granulated sucrose and powdered milk to provide laboratory cultures for various experiments.

Experimental Procedures

Experiment I: Incubation of egg at different temperature.

To study the incubation period of the egg (i.e. time from oviposition to emergence of the first instar larvae), gravid females of *L. sericata* or *C. albiceps* were allowed to oviposit on minced meat placed in black 35-mm height film cups. This provided a dark and moist environment preferred by female flies for oviposition. To ensure that eggs are of the same age, they were collected within 30 minutes of oviposition, so that they would be the same age. Clumps of eggs were separated by a gentle shaking for 10 minutes in a tube containing 0.1 M NaOH solution (Sanderman *et al*., 1987). Unseparated floating eggs and the solution were decanted. The remaining eggs were rinsed twice by filling the tube with distilled water, which then was decanted when the eggs had settled. The eggs were poured on filter paper in Petri dishes, which was kept moist throughout the experiment. The resulting egg-monolayer facilitated recognition of larval emergence. Petri dishes with eggs were put in the incubator at

one of the four different temperatures (17, 23, 29 or 35 ± 0.5 ºC). The Petri dishes were checked under a dissecting microscope at two hours intervals until the diagnostic markers of hatching became visible (Greenberg, 2002). Observations were then made at 15 minutes intervals.

Experiment II: Development at different temperatures.

Eggs were collected within 30 minutes of oviposition as mentioned in experiment I. Containers used in these experiments were one-litre jars. Each jar was filled, in the following order, 5 cm height with moistened sawdust, 140 gm of minced meat, samples of about 100 eggs, and dry sawdust to within 2 cm of the top. The mouth of the jar was then covered with a fine mesh cloth and sealed with a rubber band. Each jar was placed in an incubator at various temperatures (17, 23, 29 or 35 ± 0.5 ºC).

The feeding period of larvae was recorded as the time from emergence to when the first individual of the third instar larva stopped feeding, and left the meat for the dry sawdust. To estimate the time an observation schedule of 6-h intervals was used until the two larval moults occurred and then was followed by a schedule of 2-h intervals. The duration of postfeeding larval stage was estimated from the onset of feeding cessation to the onset of pupation. Pupae were collected and held for adult eclosion. Both pupation and adult eclosion were observed periodically at 6-h interval. This procedure was repeated three times for each species at each of the selected temperature values.

Developmental threshold and thermal constant

Lower threshold temperature (t_L) for development was estimated from the linear regression of the development rates (y = 1/developmental time) at constant temperature (x) (Campbell *et al*., 1974 and Tun-Lin *et al*., 2000). The thermal constant (K) was calculated from the equation $K = y\ (t - t_L)$, where y is the development time (days), t is the rearing temperature (ºC) and t_L is the theoretical lower development threshold temperature (ºC). Values of K represent the number of degree days (DD) above the threshold (t_L) needed for the development of the larvae and total development.

Results

Development at different temperature regimes

The mean minimum duration of each development stage and the minimum duration of total development of *L. sericata* and *C. albiceps* at the four constant temperature values (17, 23, 29 and 35ºC) are given in Table 1. The percentage of time taken by each stage is also given in the table. The minimum duration of the egg incubation and larval stage of *L. sericata* were respectively 3.75-5.69 % and 42.70-51.45% of the total development time, whereas that of *C. albiceps* were respectively, 4.92-5.78 % and 55.6-60.0%. The minimum duration of pupal stage in *L. sericata* is as long as that

of total larval development. Compared with *C. albiceps*, the duration of the pupal stage is less than that of the larval stage. Overall, the durations of the different stages of development decreased as the rearing temperature increased.

Development threshold and thermal constant

The relationship between the development rate of each species and rearing temperature is shown in Fig. 1. The larval development rates for *L. sericata* are 0.0666, 0.1401, 0.1757 and 0.2183 at 17, 23, 29 and 35ºC, respectively. The equation of the regression line is $y = 0.0083x - 0.0651$ ($R^2 = 0.9712$). The minimum threshold for the larval development is 7.8ºC and the thermal constant is 122.94±12.10 degree-days. The total development rates are 0.0279, 0.0726, 0.0854 and 0.0998 at 17, 23, 29 and 35ºC, respectively. The linear regression of development rate from egg to adult is $y = 0.0038x - 0.0276$ ($R^2 = 0.9016$). The minimum threshold for the total development is 7.3ºC and the thermal constant is 274.80±6.30 degrees-days.

For *C. albiceps*, the larval development rates are 0.0535, 0.1126, 0.1488 and 0.1905 at 17, 23, 29 and 35ºC, respectively. The equation of the regression line is $y = 0.0075x - 0.0675$ ($R^2 = 0.9884$). The minimum threshold for the larval development is 9ºC and the thermal constant is136.18±10.3 degree-days. The total development rates are 0.0318, 0.0628, 0.0827 and 0.1145, respectively at 17, 23, 29 and 35ºC. The linear regression of development rate from egg to adult is $y = 0.0045x - 0.0432$ ($R^2 = 0.9927$). The minimum threshold for the total development is 9.6ºC and the thermal constant is 225.57±9.94 degree-days.

The data in Table 1 were used to construct the isomorphen diagram for both species (Fig. 2). In this diagram, the time from oviposition to eclosion is plotted against temperature, each line representing developmental changes. Areas between lines represent identical morphological stages of both species. This diagram is especially useful when postfeeding larva or pupa of each species is recovered from the corpse, a condition at which weight is no longer a useful criterion of age.

Discussion

Since blow flies, such as *L. sericata* and *C. albiceps*, are forensically important in determining the time of death of a victim, their biology and development have been intensively studied. Results from previous researches conducted in various regions of the world on the development of both species were different from those recorded in the present investigation (Marchenko, 1985; Wall *et al.*, 1992b; Anderson, 2000; Grassberger and Reiter, 2001).

The development of insects depends on numerous factors including the experimental conditions of rearing. The kind of larval medium used could explain the different duration of observed development times. Recent studies by Kaneshraja and Turner (2004) indicated that the larval development of *C. vicina* was significantly faster by as much as 2 days on pig's lung, kidney, heart or brain tissue compared with liver.

Similar results were obtained by Clark *et al.* (2006) who found that larvae of *L. sericata* developed significantly faster when reared on lung and heart compared to liver. The development time obtained for *C. albiceps* at the four temperature regimes were slower when compared with an earlier research conducted in Russia on development of *C. albiceps* by Marchenko (1985). The differences observed in the development of larvae, reared on the same larval medium, suggests that the geographic origin of the populations could be considered as another factor that may also partly explain these differences. Greenberg (1991), Anderson (2000) and Grassberger and Reiter (2002 a, b) contended that necrophagous species of the fly presented geographic variation and adaptation. Their development times may differ under the same developmental conditions according to the population studied.

The present study shows that, at 17ºC, *L. sericata* required 859 h for total immature development, compared to 787 h (Wall *et al.,* 1992b) and 842 h (Grassberger and Reiter, 2001) in studies conducted at the same temperature for the same species from UK and Vienna, respectively. This indicates poor cold adaptation to cold conditions in our Palaearctic regional strain. The faster development of *C. albiceps* reported by Marchenko (1985) also reveals a greater adaptation to the temperate climate in the strain studied by him compared to the strain existing in our Palaearctic Region.

Under the same rearing conditions, the duration of the larval development of *C. albiceps* could be longer than that of *L. sericata*. The development threshold of *C. albiceps* is higher than that of *L. sericata*. These results suggest that *C. albiceps* requires milder temperature conditions to develop properly as *L. sericata*.

Haskell *et al.* (1997) pointed out that entomological evidence found on and around the corpse should be collected and preserved according to medico-legal standard procedures. As assumed by Grassberger and Reiter (2001 and 2002b), larvae or pupae recovered from the scene should be stored at a constant temperature, until they pupate or the first adults emerge. Their age can then be determined retrospectively, using the isomorphen-diagram. As the regional studies may be desirable to increase the precision of PMI estimates in local medico-legal investigations (Greenberg, 1991), isomorphen-diagram constructed from data recoded for each region is very useful to provide a quick and precise minimum estimate for the PMI.

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Table 1. Mean minimum development time (in hours) and percentage of development of *Lucilia sericata* and *Chrysomya albiceps* life stages raised at different temperature regimes.

Stage	Mean minimum development time			
	17ºC	23ºC	29ºC	35ºC
	Mean ± S.E. %	Mean ± S.E. %	Mean ± S.E. %	Mean ± S.E. %
Egg	32.22±0.28 3.75	18.54±0.20 5.69	13.03±0.17 4.65	10.03±0.18 4.17
	41.33±0.58 5.47	18.51±0.08 4.92	14.41±0.08 4.97	12.11±0.03 5.78
Feeding larva	222.56±0.93 25.91	79.34±0.22 23.31	73.11±0.63 26.07	61.33±0.61 25.47
	328.57±0.66 43.57	132.51±0.10 34.68	89.31±0.34 30.77	70.53±0.12 33.65
Postfeeding 3rd instar	144.00±0.10 16.76	92.17±0.13 28.14	63.59±0.70 22.82	48.55±0.27 20.33
	120.03±0.31 15.91	80.50±0.05 21.11	72.08±0.05 24.85	55.39±0.20 26.43
Pupa	460.56±0.10 53.59	140.42±0.09 42.86	130.27±0.24 46.46	120.50±0.25 50.03
	264.55±0.14 35.06	150.25±0.10 39.29	114.28±0.36 39.41	71.56±0.10 34.14
Total	859.34±0.7	330.46±0.18	281.00±0.40	240.41±0.87
	754.48±0.21	382.17±0.20	290.08±0.05	209.58±0.17

Upper figures for *Lucilia sericata*.
Lower figures for *Chrysomya albiceps*.
Data obtained from 3 replicate rearings.