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Egg Developmental Time and Survival of *Chrysomya megacephala* and *Chrysomya putoria* (Diptera: Calliphoridae) Under Different Temperatures

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ABSTRACT *Chrysomya megacephala* (F.) and *Chrysomya putoria* (Wiedemann) (Diptera: Calliphoridae) are considered of forensic, medical, and veterinary importance in Brazil because of their necrophagous and synanthropic behaviour. The development of flies can be influenced by temperature, and species from the same genus usually have different responses to external variables. The egg development of blow fly can be a useful complementary technique to estimate the minimum postmortem interval. Thus, this study aimed to compare the egg developmental time and survival of *C. megacephala* and *C. putoria* at different temperatures to determine the optimal temperature for egg development and the linear regression for developmental time and temperature, thereby determining the minimum threshold (t) and thermal summation constant (K) for each species. Adults of both species were collected in the region of Campinas city, São Paulo state, Brazil. Eggs were incubated at eight constant temperatures between 05 ± 1°C and 35 ± 1°C and the egg developmental time and survival were evaluated. There was no egg survival at 5 and 10°C. The K for *C. megacephala* and *C. putoria* were 179.41 HD and 189.94 HD, respectively. The regression slopes and t (10°C) were similar for both species. The optimal temperature for egg survival was between 25 and 35°C, for *C. megacephala* and 20 and 30°C, for *C. putoria*. The present data were similar to most data available in the literature, but differences in the same species are a possibility.

KEY WORDS blowfly, development, threshold, necrophagous

Chrysomya megacephala (F., 1794) (Diptera: Calliphoridae) is attracted by carcasses of mammals and birds and human faeces (Prins 1982) for oviposition (D'Almeida 1988). Adults of *Chrysomya putoria* (Wiedemann, 1830) (Diptera: Calliphoridae) are commonly found in latrines and cesspits and breeds in poultry dung (Conway 1972, Hulley 1983, Rognes and Paterson 2005). Both species can be found breeding in animal carcasses (Guimarães et al. 1978, Rognes and Paterson 2005) and have also been reported as mechanical vector of several viruses, bacteria, protozoan cysts, and other enteric pathogens (Greenberg 1971, 1973; Guimarães et al. 1978), occasionally causing myiasis in traumatic lesions of animals, including humans (Zumpt 1965, Guimarães et al. 1978, Ferraz et al. 2010), and infesting foodstuff (Guimarães et al. 1978). These species were also reported colonizing corpses in the Brazilian States of Paraíba, Pernambuco, Rio de Janeiro, and São Paulo (Carvalho et al. 2000, Oliveira-Costa et al. 2001, Andrade et al. 2005, Oliveira and Vasconcelos 2010). Therefore, they are considered of forensic, medical, and veterinary importance in Brazil.

The development of Diptera species can be influenced, for example, by temperature, relative humidity, photoperiod, and latitude (Wells and Kurahashi 1994, Mello et al. 2012, Nassu et al. 2014). Studies have also demonstrated that species of the same genus can exhibit different developmental rates even under similar rearing conditions, such as temperature and/or the presence of drugs (Lefebvre and Pasquerault 2004, Sukontason et al. 2008, Niederegger et al. 2013, Rezende et al. 2014). In the medical–legal context, the developmental parameters of flies are used mainly for calculating the postmortem interval (PMI) (Greenberg 1991, Catts and Goff 1992). The minimum postmortem interval (PMI_{min}), time between the beginning of body colonization by insects and the discovery of the corpse (Catts and Goff 1992), can be calculated using linear models of development (e.g., Wagner et al. 1984, Ikemoto and Takai 2000).

Developmental rates of insects at different temperatures have been studied for forensic purposes to improve the accuracy on the PMI_{min} estimative (Amendt et al. 2004). Temperatures above or below the temperature threshold inherent to each species can delay the egg incubation time or disrupt, even temporarily, the development of the immatures by interfering with their physiological processes (Wigglesworth 1972, Richards

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et al. 2009a) and, consequently, affect the egg survival (Yang and Shiao 2014). Considering that, generally, blow fly species arrive and lay eggs within few minutes after the death (Catts 1992, Campobasso et al. 2001), the use of egg developmental time can be a useful complementary technique to estimate the time elapsed from the death until the discovery of the body (VanLaerhoven and Anderson 2001, Bourel et al. 2003, Tarone et al. 2007), especially in cases of early deaths (VanLaerhoven and Anderson 2001). In this way, the demand for studies of blow fly egg developmental time under different temperatures, for forensic application, is remarkable. Thus, this study aimed to compare the egg developmental time and survival of *C. megacephala* and *C. putoria* at different temperatures to determine the optimal temperature for egg development and the linear regression for developmental time and temperature, thereby determining minimum threshold (t) and thermal summation constant (K) for each species and to compare these parameters with the data available on the literature.

Materials and Methods

Collection of Flies and Colonies Establishment in the Laboratory. Adults of *C. megacephala* and *C. putoria* were collected in the metropolitan region of Campinas city (22° 54′21″ S, 47° 03′39″ W), State of São Paulo, Brazil. *C. megacephala* was collected in an urban area, using chicken gizzards and rotten ground beef as baits, while *C. putoria* was collected in the vicinity of a poultry farm, both with the aid of an entomological net. Specimens were placed in freezer (−20°C) for 3 min to proceed trial and identification, using an interactive taxonomic key (Grella and Thyssen 2011). Then, the species of interest were kept in plastic cages with water ad libitum, sugar, and protein, at controlled temperature (25 ± 1°C), humidity (70 ± 10%), and photoperiod (12:12 [L:D] h), to establish colonies.

Egg Developmental Time Development. For the experiments, six cages of adult flies of each species were used. Four small Petri dishes without the lids, with 2-cm-diameter liver beef pieces each, were put in each cage as oviposition substrate and observed every 30 min. The Petri dishes with an egg mass with ~0.5 cm of diameter were removed from the cages and inserted in larger Petri dishes with lids to prevent hatched larvae to escape. The closed Petri dishes were placed on growth chambers (Model 202/4, Eletrolab, São Paulo, São Paulo) with controlled photoperiod (12:12 [L:D] h) and constants temperatures of 5, 10, 13, 17, 20, 25, 30, and 35 ± 1°C. This procedure was repeated until there were four replicates for each species and temperature. The replicates were placed in the same growth chamber and ran simultaneously. The eggs were not manipulated to prevent any interference on the egg survival; therefore, their counting were performed only after the larval hatching. The Petri dishes were also observed every 30 min until the beginning of larval hatching or up to 168 h, if no larval hatching was observed. The Petri dishes without egg survival were discarded without counting the eggs.

Egg Survival. After 5 h from the beginning of hatching, the Petri dishes were sealed with Parafilm M and stored in freezer. For counting the larvae and chorions, the Petri dishes were removed from the freezer and let untouched until they reached room temperature, then the egg masses were separated with a soft thin brush and saline solution to proceed the counting. Both the larvae that had successfully hatched and the remained eggs were counted with the aid of a stereomicroscope (Model Stemi SV 11, Carl Zeiss, Oberkochen, Baden-Württemberg, Germany) and the egg survival was calculated using the equation: hatched larvae/(hatched larvae + remained eggs).

Data Analysis. The analysis of covariance (ANCOVA) test (PROC GLM, SAS Institute 2009, Cary, NC) was used to compare the regression slopes of the two species, data were analysed using SAS (Statistical Analysis System; SAS 2006) software with an overall error rate (α) of 0.05. Quadratic regression (Crawley 2007) was used to indicate the optimum temperature for egg survival and Mann–Whitney U-test (Crawley 2007) was used to compare the egg survival of both species in each temperature, using R Core Team (2013) system (Vienna, Austria).

For comparison of the data collected in this paper concerning developmental time versus temperature and the data pooled from literature, a graphic was made using Excel 2013.

Linear Model. The linear model used to determine the Accumulated Degree Hours (ADH) for the egg developmental time was calculated using the equations according to Ikemoto and Takai (2000) Method 2: $(DT) = K + tD$, which relates duration of development (D) in hours, temperature of development (T) in degrees, minimum developmental threshold (t) in degrees, and thermal summation constant (K). In the figures, the lines represented by this equation have $x = D$ and $y = DT$. The calculus and figures were made using SAS (Statistical Analysis System; SAS 2006).

Results

The mean number of eggs per temperature ranged from 100 to 867 for *C. megacephala*, from 89 to 743 for *C. putoria*, and there was no hatching recorded at 5 and 10°C (Table 1). The egg survival was higher between 25 and 35°C for *C. megacephala* and between 20 and 30°C for *C. putoria* (Fig. 1) and was different between the species only at 20°C ($P = 0.0294$).

The relation between egg developmental time and temperature did not differ between both species, according to ANCOVA test ($P = 0.7813$; $R^2 = 0.754$; SD = 1.38). For both species, equations of the development were calculated assuming that the relationship between the time of development and temperature is linear. The curvatures on temperatures above and below thresholds were considered, but all points were part of the linear relationship. For *C. megacephala*, the equation was $y = 179.41 + 10.82x$; $R^2 = 0.972$ (Fig. 2), and, according to that, $t = 10.8$°C (SE = 0.82) and $K = 179.41$ HD (SE = 26.69). For *C. putoria*, the equation was $y = 189.94 + 10.29x$; $R^2 = 0.997$ (Fig. 2),

Table 1. Mean number of eggs ± SD, incubation time (hour), and egg survival (%) of *C. megacephala* (F.) and *C. putoria* (W.) (Diptera: Calliphoridae) at eight temperatures, where NA = not applicable

Temp (°C)	*C. megacephala*			*C. putoria*		
	No. eggs ± SD	Duration of development (h) ± SD	Egg survival (%) ± SD	No. eggs ± SD	Duration of development (h) ± SD	Egg survival (%) ± SD
5	NA	NA	0	NA	NA	0
10	NA	NA	0	NA	NA	0
13	818 ± 89	64.0 ± 1.4	22.7 ± 6.3	436 ± 343	69.0 ± 2.1	15.4 ± 8.2
17	867 ± 254	39.4 ± 8.5	22.6 ± 28.0	743 ± 437	28.4 ± 0.3	64.4 ± 20.8
20	205 ± 59	21.1 ± 0.6	66.2 ± 10.7	89 ± 63	21.0 ± 0.0	90.2 ± 7.6
25	142 ± 72	12.8 ± 0.0	84.8 ± 14.1	137 ± 86	13.0 ± 0.4	68.1 ± 19.1
30	100 ± 51	8.4 ± 0.3	80.8 ± 12.6	269 ± 161	8.6 ± 0.4	83.4 ± 12.8
35	125 ± 61	6.5 ± 0.0	82.9 ± 12.6	401 ± 545	8.0 ± 0.6	63.5 ± 17.6

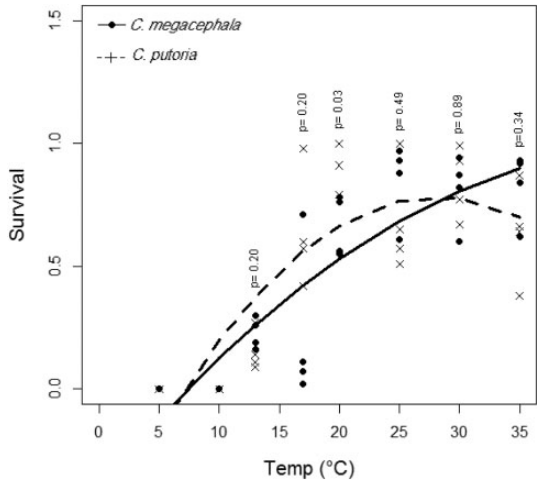

Fig. 1. Egg survival for *C. megacephala* (F.) and *C. putoria* (W.) at eight temperatures. The equations that represents the survival are, for *C. megacephala*: $y = -0.4021 + 0.0590x - 0.0006x^2$; $R^2 = 0.75$, and for *C. putoria*: $y = -0.6293 + 0.1002x - 0.018x^2$; $R^2 = 0.68$. The *P*-values are based on the Mann–Whitney test for comparisons of the egg survivor of the two species in each tested temperature.

$t = 10.3°C$ (SE = 0.25), and $K = 189.94$ HD (SE = 8.21).

The egg developmental time decreased with the temperature increase, as expected, varying from over 64 h at 13°C to 7 h at 35°C, for *C. megacephala*, and, for *C. putoria*, between 69 h at 13°C and 8 h at 35°C (Fig. 3). The egg developmental time for *C. megacephala* was similar to the data available on the literature, restricted to temperatures ~26°C for populations from South Africa (Prins 1982), India (Wells and Kurahashi 1994), and Brazil (Barros-Cordeiro and Pujol-Luz 2010), but diverged of a population from Egypt (Gabre et al. 2005) (Fig. 3). For *C. putoria*, the egg developmental time was similar to the 15.5 h presented by Greenberg and Szyska (1984), if the mean temperature of development considered is 23.9°C (higher and lower temperatures during the development of 21.7 ± 1.9 and 26.0 ± 3.1°C, respectively).

Discussion

The thermal requirements achieved for the egg development differ from those present in the literature for the adults of *C. megacephala* and *C. putoria*, although it was expected this would not vary once the metabolism kinetics tend to be constant at all insects stages (Sharpe and DeMichele 1977). Richards et al. (2009a) observed that the thermophysiological thresholds for the adults of *C. megacephala* and *C. putoria* were ~21 and 24°C, respectively. An average minimum developmental threshold for adults of 10.40°C (experimental data) and of 14.68°C (pooled data from the literature), besides an upper critical temperature of ~35°C (experimental data) for *C. megacephala* were provided by Richards and Villet (2009). For *C. putoria*, the minimum developmental threshold estimated by Richards et al. (2009b), considering all developmental landmarks, except egg developmental time, was of 13.42°C, and the upper critical temperature of ~49°C for third-instar maggots (Richards et al. 2009a).

Wells and Kurahashi (1994) determined *C. megacephala* egg developmental time between 12 and 18 h at 27°C, Prins (1982) and Barros-Cordeiro and Pujol-Luz (2010) determined a duration of 14 and 15 h, respectively, at 26°C, and Richards and Villet (2009) observed egg developmental time between 19 and 21 h for 22°C, all somehow similar to the results presented here for 20°C (21 h) and 25°C (12.5 h). However, the development presented by Gabre et al. (2005) of 24 h at 26°C for a population from Egypt was twice the time recorded in other studies. The *t* determined here for *C. megacephala* egg developmental time was lower to the one estimated by Richards and Villet's (2009) compilation of 12.26°C, as to the $K = 195.8$ HD from their pooled data. Lefebvre and Pasquerault (2004) pointed out the importance to consider that same species can present different developmental time depending on their geographic region, due to adaptive changes triggered by environmental characteristics.

For *C. putoria*, egg developmental time data of Greenberg and Szyska (1984) was of 14.5 and 16.5 h for two groups of eggs exposed to temperatures that fluctuated between 21.7 and 26.0°C. These data can be similar to the one presented at 25°C (13 h) if the temperature of development considered is the mean

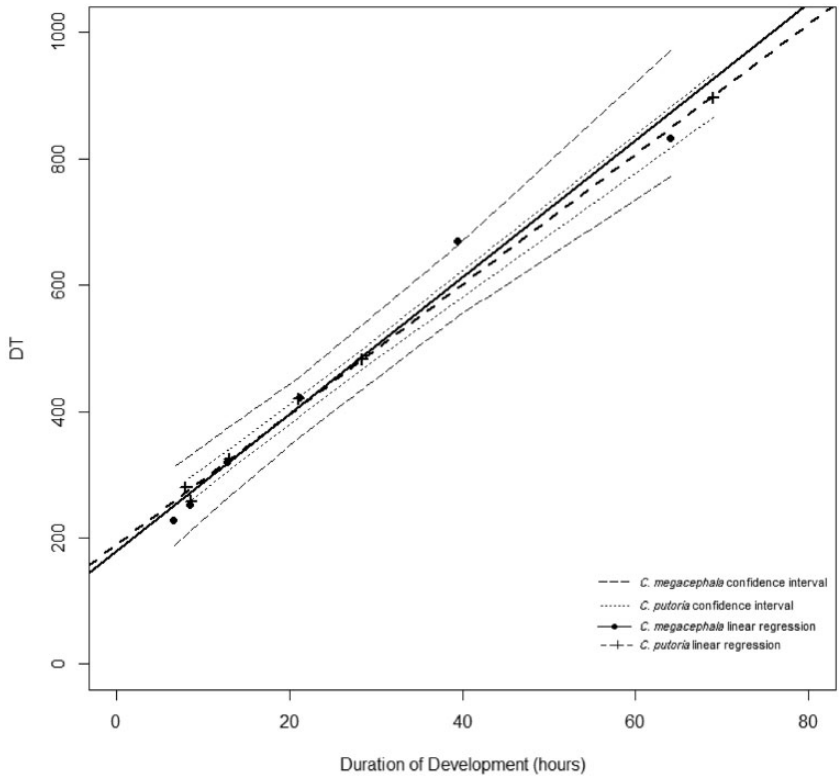

Fig. 2. Temperature (T) and duration of development (D) of *C. megacephala* (F.) and *C. putoria* (W.). The regression lines are used to determine *t* and *K* for egg development for each species.

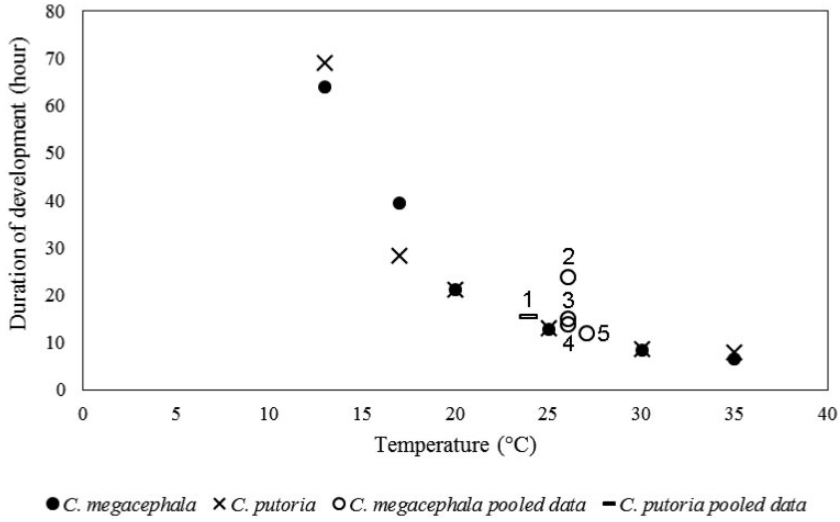

Fig. 3. Developmental time at different temperatures for *C. megacephala* (F.) and *C. putoria* (W.) data here presented and published data. 1, Greenberg and Szyska 1984; 2, Gabre et al. 2005; 3, Prins et al. 1982; 4, Barros-Cordeiro and Pujol-Luz 2010; 5, Wells and Kurahashi 1994.

temperature. Thought fluctuating temperatures might retard or speed the insects' development (Greenberg 1991), Anderson (2000) asserted that the error caused by the use of the duration of development data under constant temperatures can be conservative for the PMI_{min} estimate. In addition, our results showed no differences between the slopes of *C. megacephala* and *C. putoria*, indicating there is no need of doing the egg

differentiation between these two species to use these data on the PMI_{min} estimate based on egg development for the region of Campinas city.

The egg developmental time of *C. megacephala* and *C. putoria* decreased with the increasing of the temperature, as observed in the Greenberg and Kunich's (2002) compilation for another Calliphoridae species. In the same way, the egg survival of both species was higher with the increasing of the temperature, as previous recorded for *C. megacephala* by Yang and Shiao (2014). The higher egg survival for *C. megacephala* between 25 and 35°C and for *C. putoria* between 20 and 30°C are in accordance to the expected. Yang and Shiao (2014) obtained the highest values of *C. megacephala* egg survival at 20 and 25°C.

In Campinas, between 1998 and 2008, the annual average temperature was of 22.4°C and the hotter and colder months had a difference of 6.4°C between average temperatures (Cepagri 2015). The minimum average of July was of 12.3°C (Cepagri 2015), when eggs of *C. megacephala* and *C. putoria* would take 64 and 69 h to develop and only 22 and 15% of eggs would survive, respectively. While in February, the maximum average was of 30°C (Cepagri 2015), so the *C. megacephala* and *C. putoria* egg developmental time would be of 8.5 and 8.6 h and egg survival of 80 and 83%, respectively.

Sukontason et al. (2008) studied *C. megacephala* and *Chrysomya rufifacies* (Macquart) development under natural temperatures in Thailand (averages between 18.4 and 31.4°C in the studied year), observing the egg developmental time of 12–24 h, suggesting the addition of 24 h in the Thailand mean temperatures for corresponding to the embryonic development. This recommendation should not be applied to the PMI_{min} estimate based on the egg developmental stage in view of our results, which pointed out that the developmental time of the egg is temperature dependent and might be known for the PMI_{min} estimate accuracy, as stressed by VanLaerhoven and Anderson (2001).

As described in Greenberg's (1991) and Anderson and Cervenka's (2002) case reports, the data presented for *C. megacephala* and *C. putoria* contribute with useful information for the PMI_{min} estimate based on the egg developmental stage for Campinas city, and improve the knowledge of natural history of these Calliphoridae species, providing new data about their biological features.

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