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Development Time Variability: Adaptation of Régnière's Method to the Intrinsic Variability of Belgian Lucilia Sericata (Diptera, Calliphoridae) Population

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Abstract

Development times and survivorship of *Lucilia sericata* (Meigen, 1826) were measured for different constant temperatures. In addition, lower threshold (tL) and thermal constant (Kt) were estimated for a local population. The estimated development time calculated from mean or minimum development time induces a bias that may be reduced using a model of intrinsic variability. In order to quantify variation in emergence rate during the development time, a simulation method was developed by adapting the Régnière's method. The adapted model allowed accurate prediction of the emergence profile of *L. sericata* at constant temperature in the linear portion of the development curve. We believe that the application of this method in forensic entomology cases could increase the reliability of the conclusions of an entomological Post Mortem Interval (PMI) estimation.

Keywords: Blowflies; Forensic science; Development speed; Mortality

Introduction

Development duration of *Lucilia sericata* (Meigen, 1826) (Diptera: Calliphoridae), as for many other insects, depends on temperature. This relationship is curvilinear at low and high temperatures and linear in-between [1]. However, even at a chosen temperature, a large variability in development times is observed within published data [2-6].

This variability is largely due to inter-individual competition [7, 8], food type [9, 10] or nyctemeral rhythm [6, 11]. Such variability could also result from different experimental designs [6, 11]. Furthermore, the given development time are mainly based on summary statistics such as minimum [3, 12], median [5, 13, 14], mean [2, 15], maximum [3] or percentage [1, 4, 16]. The use of these different sampling methods and types of synthetic parameters could bias the accuracy of the final published development data [14]. Therefore, Wells and Lamotte [17] promote the use of full distributions instead of summary values.

To include this intrinsic variability in development-time duration estimation, Régnière [18] describes a method based on a correction factor handset to median developmental value. Interestingly, median is an often used synthetic value and is recommended by Richards and Villet [14] in the context of forensic entomology. A more recent publication suggests a parameter estimation procedure for time distributed delay (TDD) models [19]. But this process is, however, limited to symmetric development distributions [19].

The present study estimates the survivorship and summary values of development for a Belgian *Lucilia sericata* population at different constant temperatures. Using this dataset, we described an adaptation of Régnière's method to simulate intrinsic variability on *L. sericata* development speed. The adapted method has also been used to simulate emergence curves from experimental data.

Material and Methods

Experimental data

Larvae of the blowfly *Lucilia sericata* were collected from a field in Brussels (National Institute of Criminology and Criminalistics, Brussels, Belgium). The flies were held in an insectarium at 24 ± 2°C with 70 ± 5% relative humidity (RH) and a photoperiod of L16:D8 and fed *ad libidum* with water, sugar, powdered milk and brewer's yeast.

For oviposition, a piece of beef heart was provided within 4 hours. The time when the beef heart was removed from the cage was recorded as the beginning of development [16]. Eggs batches were collected from this piece and about 200 eggs were deposited on 250g of beef heart in a plastic box [16]. Experiments were conducted in incubators (Sanyo, Incubator MIR 553) set at constant temperatures of 12.5, 15, 20, 25, 30, 35 or 37.5 ± 1°C with a photoperiod of L12:D12. A data logger (Testo, 174) was inserted into the incubators to monitor the temperatures each hour. This procedure was repeated at minimum 8 times for each temperature regime except for mortality at 20°C. Successfully emerging adults were recorded daily and removed from the box [14].

Description of the analytical method

Reduced major axis regression [20] was used to calculate lower

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Temperature (°C)	No of repetitions (and pupae)	Emergence (%)	Standard Error (%)
12.5	12 (1366)	22.8	12.7
15	18 (1171)	44.3	12.1
20	4[a] (583)	65.9	27.4
25	12 (2389)	85.7	10.7
30	10 (2061)	95.4	6.6
35	12 (2754)	40.7	14.8
37.5	8 (579)	**no emergence**	

[a]Percentage of emergence was counted only on 4 treatments and not for the totality of treatments at 20°C

Table 1: Percentage of pupae to successfully emerge as adults of *Lucilia sericata* reared under constant temperatures and photoperiod of L: D 12: 12 h.

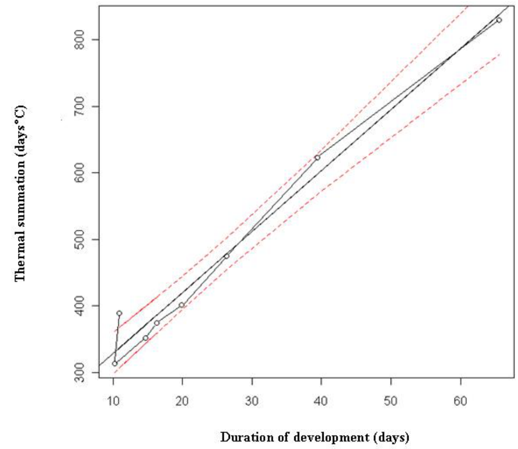

Figure 1: S-shaped curves and reduced major axis regression lines used to determine Kt and t_L values of *Lucilia sericata* at 6 constant temperatures for emergence. White and black circles indicate respectively median development time used and not used in the regression calculations and dotted lines represent 95% confidence intervals.

threshold (t_L) and thermal constant (Kt) from the median development time at each temperature [13, 14].

In Régnière's method, the proposed distribution function is polynomial. However, in this study, we have used Weibull distribution function [21], which allows equivalence between the mean and the median. Thus, most of published data from previous forensic entomology studies could be used. Two-sample Kolmogorov-Smirnov tests were applied to test the reliability between emergence profiles of *L. sericata* observed experimentally and based on Régnière's simulations. All analyses were conducted using open source software R-2.8.1.

Results

Standard development of population

Between 12.5 and 30°C, the percentage of emergence increased with increasing temperature. At 35°C, the percentage of emergence starts to decrease, and finally at 37.5°C no pupae eclosed (Table 1). In this temperature range (12.5-30°C), the median development times of *Lucilia sericata* have a linear relationship with temperature ($R^2=0.99$) (Figure 1). Development times (days) of *Lucilia sericata* decreased with higher temperatures. The lower threshold (t_L) for total development is 9.55 ± 0.41 (SD)°C and the thermal constant (Kt) for *Lucilia sericata* was 217.97 ± 15.01 (SD) Degree Days above the threshold.

Intraspecific variability modelisation by adapted Régnière's method

According to Régnière's recommendations, population was divided for each temperature into sub-populations, each corresponding to an equal part of the whole population. Thus, the Weibull distribution law's fitting parameters (W, K and Q) and statistics were determined and are summarized in Figure 2. Finally, replacing W, K and Q in Régnière's equation gives the correction factor P to apply for each percentile of population.

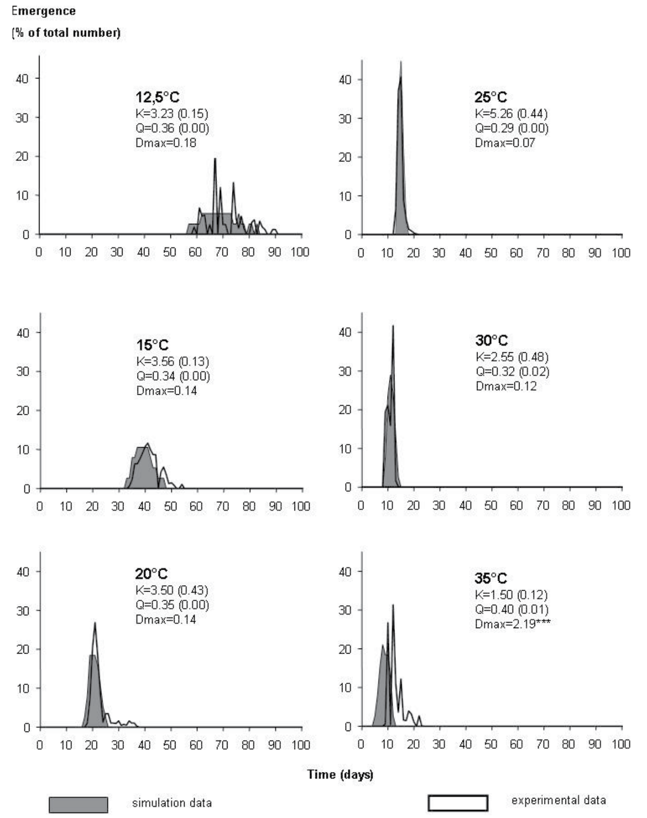

Figure 2: Comparison at different constant temperatures between profiles emergence of *L. sericata* observed experimentally and in simulation. Asterisks (Kolmogorov-Smirnov ***p<0.001) indicate profiles emergence that differ statically between observed and simulated data. Parameters K and Q are indicated in each figure with standard error and statistics. W is indeed constant and thus independent of temperature.

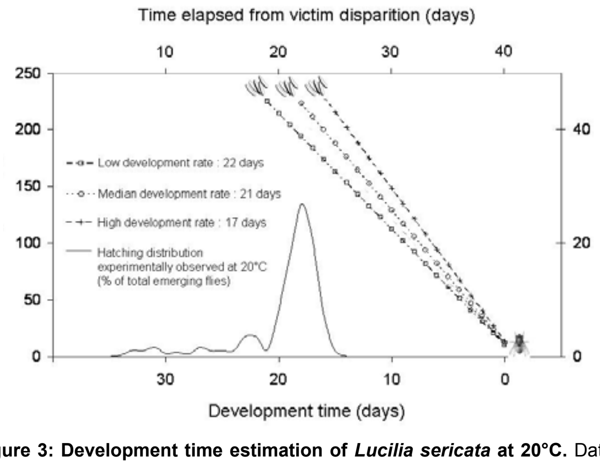

Figure 3: Development time estimation of *Lucilia sericata* at 20°C. Dates were calculated from hatching point (day 40) using ADD method with thermal constant (Kt, t_L) describe in present study. Effect of intrinsic variability on development time is estimated using a weighting factor (P) calculated from experimental data.

Then, we multiply the median accumulated degree-days (ADD) by weighting factor (P) relative to each sub-population and temperature to obtain weighted value (ADDw = ADD x P). As a result, the curve of emergence could be rebuilt by addition of these values until Σ ADDw=Kt. Only at 35°C, the emergence profiles differ statistically between observed and simulated data.

The usefulness of this adapted method is demonstrated by forensic entomological example. We considered the case of an adult *L. sericata* emerging on day 40 at ambient temperature (Figure 3). Using minimum development times, oviposition will be placed on day 20. However, this emergence can also result from oviposition at day 19 until day 12 if respectively the median or the maximum development times are used. The probability of each one of these hypothesis can then be determined from the sampling rate.

Discussion

According to Wall et al. [7], the percentage of emergence is temperature dependant and extremely variable, ranging from 0 to 96%. By contrast of the present study, emergences of adults were observed at 15°C by Grassberger and Reiter [4].

Analysis of these developmental data revealed that emergence profiles of single population at different constant temperatures demonstrate a large variability, due to a different individual development rate. Unfortunately, this intrinsic variability is not-well mentioned and studied in forensic studies. The proposed model describes the intrinsic variability of a local population and can accurately predict the emergence profile of *L. sericata* at constant temperatures.

In addition, this method could be applied for forensic PMI estimations. Thus, a complete interval for the PMI estimation can be obtained, which is of interest in forensic cases with multiple ovipositions occurring all over the time of decomposition [22]. Indeed, the best way to obtain oldest larvae (which are considered to be the most representative indicator of the first oviposition event), is gathering a large sample (*i.e.* at least 30 individuals) [23]. But in many cases, the sample is insufficient. Thus, using minimum, mean, median or maximum development time from first emergence could become tricky [22]. As an example of application, the adapted Régnière's model allows estimating the whole oviposition window susceptible to lead to an emergence point. Thus, intrinsic variability can be integrated by using this method, and so reliability of entomological expertise could be increased. However, this method must be applied in a long-term study including different experimental and human cases to confirm increase of reliability.

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