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Influence of constant and changing temperatures on the larval development of *Calliphora vicina* (Diptera: Calliphoridae)

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Abstract. Observations on the blowfly *Calliphora vicina* Robineau-Desvoidy, 1830 under quasi-natural conditions in the environs of St. Petersburg (Russia) where the average temperatures ranged from 10 to 25 °C revealed that the correlation between the rate of its larval development and the mean daily temperature could be approximated by a linear regression (R=0.89). Laboratory experiments conducted under constant temperatures of from 12 to 23 °C also yielded a strong (R=0.93) linear correlation. The parameters for the development from egg to puparium calculated based on the data recorded in the field and laboratory (low temperature thresholds of 1.1±0.7 and 2.4±1.5 °C, regression coefficients 0.55±0.02 and 0.61±0.05, respectively) were not significantly different. The sums of effective temperatures were also practically the same: 193 and 188 degree-days for field and laboratory conditions, respectively. Further increase in the constant temperature up to 25–28 °C did not result in an increase in the rate of larval development in the laboratory and was similar to the developmental rate recorded under natural conditions at mean temperatures of 24–25 °C, but were slightly lower than the regression line. The rate of larval development when subjected to an artificial thermorhythm (temperatures alternating between 22 and 28 °C every 12 hours) was not significantly different from that recorded at a constant temperature of 25 °C. We conclude that the rate of larval development of *C. vicina* under natural thermorhythms can be reliably predicted based on parameters determined in the laboratory at constant temperatures.

Key words. Temperature, thermorhythm, rate of development, larvae, blow fly, *Calliphora vicina.*

INTRODUCTION

It is well known that the rate of growth and development of insects is markedly dependent on temperature and that within the tolerance range of a species this dependence is usually close to linear. Although numerous nonlinear equations have been proposed to describe the relationships between insect development and temperature, in practice it is possible to predict the duration of insect development over a wide range of temperatures using the linear model (Honěk & Kocourek 1990, Honěk 1996, 1999, Briere et al. 1999, Jarošík et al. 2002, 2004, Trudgill et al. 2005, Bergant & Trdan 2006, Jarošík et al. 2011, Damos & Savopoulou-Soultani 2012). In addition, although certain species of insects either accelerate or retard their rate of development under varying temperature conditions, in most insects the rate of development recorded when kept in a normal thermoperiod is close to that at its mean effective temperature and, hence, the duration of their development under natural thermorhythms can be quite accurately predicted based on data obtained in the laboratory at constant temperatures (Hagstrum & Hagstrum 1970, Beck 1983, Hagstrum & Milliken 1991, Greenberg 1991, Davies & Ratcliffe 1994, Siegel et al. 2010, Warren & Anderson 2013, Wilstermann & Vidal 2013, Tu et al. 2014). The reliability of this prediction is extremely important for agriculture, forestry and many other human activities.

For the object of this study, the blowfly *Calliphora vicina* Robineau-Desvoidy, 1830, it is important to know its rate of development under constant and changing temperatures because this

insect is not only a convenient model for various laboratory studies (Vinogradova 1984, 1991) but is also widely used in forensic entomology for estimating how long a person has been dead (Vinogradova & Marchenko 1984, Greenberg 1991, Richards & Villet 2008). Thus, it is important that the determination of the duration of its development is accurate. Although, as noted above, the blowfly has long been used in various eco-physiological studies, most of these experiments were conducted under constant laboratory conditions. Recently we compared the parameters of preimaginal development of *C. vicina* recorded under natural and laboratory conditions (Vinogradova & Reznik 2013a). The results of that study indicate that the rate of development under natural thermorhythms is rather close to that recorded under corresponding constant temperatures but the data was insufficient for a reliable statistical comparison. The present paper contains the results of more detailed studies including the results of 4 seasons of field observations and numerous laboratory experiments.

MATERIALS AND METHODS

For both field and laboratory studies, the same laboratory strain of *Calliphora vicina,* which originated from flies collected in the environs of St. Petersburg, was used. Field observations were made under quasi-natural conditions during the warm seasons (May–September) of 2010–2013 in the environs of St. Petersburg, Russia. The flies were kept in gauze cages on a shaded veranda and regularly given water, sugar and protein food (pig kidneys). The batches of eggs laid during 24 h were periodically placed in 0.5-liter jars with wet sawdust and kept in a shaded place in the open air. The larvae were fed on pig kidneys. In 2010–2011 the temperature under the canopy where the jars with the larvae were kept was recorded with a mechanical temperature recorder and mean, minimum and maximum daily temperatures were calculated based on seven measurements each day at intervals of 3 h 25 min. In 2012–2013 the temperature under the canopy was recorded using a portable USB data logger every 30 minutes and the daily means, minimums and maximums were each based on 48 measurements per day. To measure the duration of larval development (from egg to puparium), the pupae were counted daily or every 2–3 days, depending on the temperature. It is well known that at temperatures lower than 16 °C some larvae of *C. vicina* enter diapause and the percentage diapausing markedly increases with decreasing temperature and is almost 100% at 8–10 °C (Vinogradova 1984, Vaz Nunes & Saunders 1989). Thus, at temperatures of 11–15 °C the temporal distribution of pupariation is strongly right-skewed or even bimodal (Vinogradova & Reznik 2013b). In the present study, only data for the "active fraction" (i.e. the larvae that pupated within 20–30 days after eclosion, depending on temperature) were used to estimate the mean duration of larval development.

Laboratory experiments were carried out during the winters of 2011–2014 in thermostatic chambers at the Laboratory of Experimental Entomology of the Zoological Institute, Russian Academy of Sciences (St. Petersburg). In the laboratory, *C. vicina* eggs were obtained from flies kept at 20 °C and an L:D=16:8h. The egg batches were divided between several jars placed in different thermostatic chambers and monitored in the same way as those reared in the field. The rate of larval development was recorded at constant temperatures of 12 and 16 °C (in the dark), 20, 23, 25, 28 °C (under a photoperiod of L:D=15:9h) and also under a thermorhythm with a mean temperature of 25 °C (12 h of 22 °C and 12 h of 28 °C) under the same photoperiod (L:D=15:9), the thermophase coincided with the middle of the photophase.

To avoid pseudoreplication, cohorts (groups of larvae that hatched from the same egg batch) were considered as statistical units both in the field and laboratory experiments (the mean duration of larval development calculated for a cohort was used). Each cohort included at least 50 larvae. In total, the development of 195 cohorts was recorded under natural conditions and 45 cohorts in the laboratory. For the field observations, the mean temperature was calculated based on the temperature records for the period of time from the day of larval eclosion to the day of mass (modal) pupation of each cohort. The statistical treatment of the data included a GLM, correlation, and regression analyses, and the regression coefficients and thresholds were compared using a Student's t-test.

RESULTS

The dependence of the rate of larval development of *C. vicina* on mean daily temperature under natural conditions was closely ($R=0.89$, $n=195$, $P<0.001$) approximated by the linear regression $Y=0.55\times X-0.62$. For the graphical presentation 195 cohorts of larvae were separated into 15 classes that experienced different average temperatures during the period of larval development: the first included cohorts that experience average temperatures of from 10 to 11 °C, the second, from

11.1 to 12 °C, etc. Most of the classes included more than 10 cohorts, while the two "extreme" classes with mean temperatures of less than 11 and more than 24 °C each included only two cohorts. The daily mean, minimum and maximum temperatures averaged for cohorts of each class are shown in Fig. 1. Although, as seen in Fig. 2, the data recorded at the highest of the average daily temperatures (24–25 °C) were somewhat lower than the regression line, this difference was not significant because of high variability and small sample size.

Laboratory experiments conducted under constant conditions also revealed that an increase in temperature from 23 to 25–28 °C caused only a slight and statistically insignificant ($R=0.32$, $n=24$, $P=0.123$) increase in the rate of larval development (Fig. 2b). Thus, only data for temperatures of less than 25 °C were used in the linear regression analysis. The correlation ($R=0.93$, $n=26$, $P<0.001$) was somewhat stronger than that recorded under natural conditions, but the parameters of the regression were similar: $Y=0.61\times X-1.48$. As seen in Fig. 2, the two regression lines are very close to one another. The parameters calculated based on these regressions (means and SE): low temperature thresholds of 1.1±0.7 and 2.4±1.5 °C, regression coefficients of 0.55±0.02 and 0.61±0.05 for larval development under field and laboratory conditions, respectively, were not significantly different (Student's t-test; $P>0.05$). The sums of effective temperatures (SET) were also practically the same: 193 degree-days in the field and 188 degree-days in the laboratory.

A two-factorial GLM analysis of the pooled data ($n=235$, temperature and laboratory vs. natural conditions were the two factors) also revealed that the rate of larval development significantly ($P<0.001$) depended on temperature, but not on laboratory vs. natural conditions ($P=0.511$). When only the data for temperatures less than 22 °C were analyzed ($n=183$), the results were the same: $P<0.001$ and $P=0.220$, but if mean temperature during larval development was higher than 22 °C ($n=52$), neither factor had a significant effect ($P=0.207$ and $P=0.278$ for temperature and laboratory

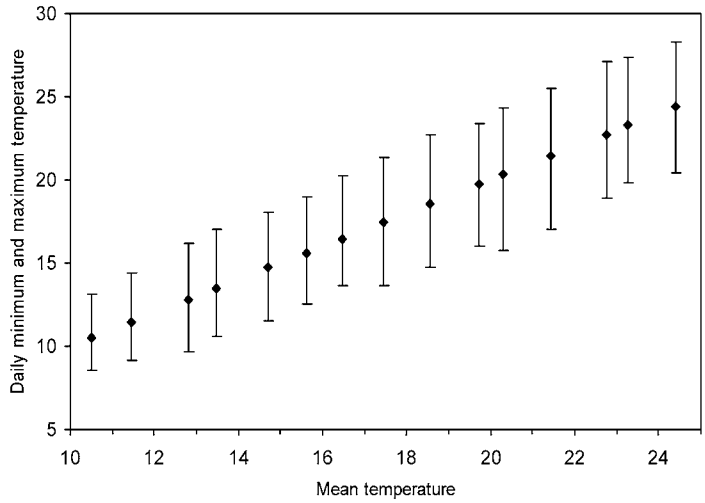

Fig 1. Average daily minimum, mean and maximum temperatures recorded in 2010–2013 under the canopy of the veranda where the jars with *Calliphora vicina* larvae were kept. Each symbol represents the data for a group of 2–28 cohorts (total n=195, the groups were separated based on the mean temperature during larval development), error bars indicate daily minimums and maximums for the same period averaged for all cohorts of a group.

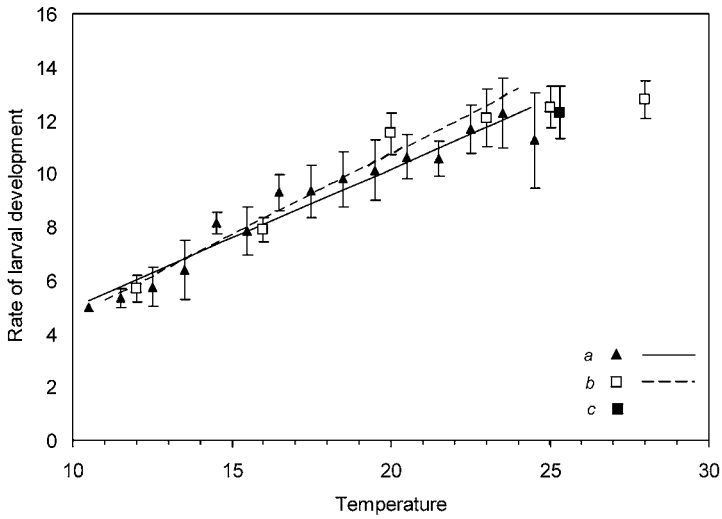

Fig. 2. The rate of larval development of *Calliphora vicina* in relation to temperature: a – under natural conditions, *b* – in the laboratory under constant temperatures, c – in laboratory under a thermorhythm of 22–28 °C with a mean temperature of 25 °C. Means and SD are indicated, data on development under natural conditions are grouped as in Fig. 1.

vs. natural conditions, respectively). Finally, the rate of larval development of *C. vicina* in the laboratory reared at an artificial thermorhythm (alternating temperatures of 22 and 28 °C, each lasting for 12 h at a photoperiod of L:D=15:9h, with the thermophase coinciding with the middle of the photophase) did not differ significantly (P=0.593, Student's t-test) from that recorded at a constant temperature of 25 °C (Fig. 2c).

DISCUSSION

Our results are similar to those of earlier studies on the blowfly by various authors (see Vinogradova 1984, 1991 for the references). For example, according to Donovan et al. (2006) SET for *C. vicina* development from egg to pupation is 195 degree-days with a threshold of 1 °C. Note, however, that our recent study (Vinogradova & Reznik 2013a) yielded somewhat different results: the SET was about 140 degree-days with a threshold of 5.8 °C. The reason for this difference is that we also included data recorded under natural conditions at mean temperatures of 5–10 °C in the analysis in our earlier study, which is lower than the upper temperature threshold for the induction of larval diapause (Vinogradova 1984, 1991, Vaz Nunes & Saunders 1989, Vinogradova & Reznik 2013a). As seen in Fig. 1a of Vinogradova & Reznik (2013a), all symbols for these results are below the regression line, which is most probably due to the induction of a short period of diapause in some individuals. Another possible reason for the very high variability of the results recorded both under natural and laboratory conditions is an uncontrolled and, most probably, density-dependent self-heating of larvae, although, for example, Davies and Ratcliffe (1994) show that larvae reared individually in small tubes grew on average at the same rate as those reared communally. On the other hand, these authors note that the variation in the rates of growth and development of *C. vicina* is generally greater than that recorded in other Diptera.

We conclude that both field observations and laboratory experiments indicate that at temperatures between 10–12 °C (which is the upper threshold for larval diapause induction) and 22–23 °C the thermal dependence can be accurately approximated by a linear regression, whereas a further increase in temperature does not cause a significant increase in the rate of larval development. This low upper limit of the optimal zone is most probably connected with the fact that *C. vicina* is a relatively cryophilous insect. In natural conditions in the environs of St. Petersburg, adults become active in April–May when mean daily temperature is 2–4 °C and terminate in summer when it reaches about 14 °C and become active again in August–September (Vinogradova 1984).

Returning to the aim of this study, we conclude that the main parameters of the thermal dependence of the rate of larval development of *C. vicina* under natural thermorhythms were not significantly different from those under constant temperatures in a laboratory. In addition, the rate of development of those exposed to the artificial thermoperiod of 22–28 °C (which is close to the average daily variation in temperature under natural conditions) was practically the same as that at the corresponding constant temperature of 25 °C. Thus, the results of our study agree with the numerous earlier investigations done using different insects, which demonstrate that the rate of development under natural thermorhythms can be quite reliably predicted based on parameters determined in the laboratory at constant temperatures. As for other Diptera, the effect of variable temperatures on the duration of development seems to depend both on the species studied and on the methods used. For example, according to the data published by various authors the rates of development of *Calliphora vomitoria*, *Protophormia terraenovae* and *Lucilia sericata* recorded at a particular thermorhythm is somewhat higher than that recorded at the corresponding constant temperature and changing the temperatures delays preimaginal development in *C. vicina*, *Chrysomya macellaria*, *C. rufifacies*, *Phormia regina*, and *Phaenicia sericata* (Hagstrum & Hagstrum 1970, Greenberg 1991, Davies & Ratcliffe 1994, Warren & Anderson 2013).

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