

Effects of temperature on the development of the Neotropical carrion beetle *Oxelytrum discicolle* (Brullé, 1840) (Coleoptera: Silphidae).

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Abstract

Due to its necrophagy, the Neotropical beetle *Oxelytrum discicolle* (Brullé, 1840) is found in association with human corpses. Its biology can provide important information to determine the postmortem interval (PMI). We studied the life cycle of *O. discicolle* in the laboratory under three constant temperatures (15, 20 and 28°C) and under natural conditions (mean T = 18.5°C) in a cloud forest in Altos de Pipe (10°20'N, 66°55'W) Miranda state, Venezuela. The total time required to complete development from egg to adult emergence shortened as temperatures increased, ranging from 40.00 ± 2.73 days at 15°C to 20.33 ± 0.89 days at 28°C. Developmental time on natural conditions was not significantly different from that obtained at 20°C. This information would be useful to forensic entomology applied to criminal investigations in Venezuela and the Neotropical region.

Keywords: *Oxelytrum discicolle*, temperature, development, forensic entomology, Venezuela.

Introduction

Necrophilous silphids have been recognized as forensically important species that colonize a body in the decay stage of decomposition [1, 2]. However, *Oxelytrum discicolle* (Brullé, 1840) is a significant component of the insect fauna associated with fresh and bloated corpses [3]. The species is distributed from southern Brazil and Paraguay, through much of central and northern South America, through Central America to Mexico [4].

This carrion beetle is the most commonly collected species of silphid in Latin America [4], and constituted a forensic indicator in the Neotropical region. In Brasil, Moura et al. [5] found adults and larvae of *O. discicolle* in rat carrion in the state of Paraná; Carvalho et al. [6] found larvae of *O. discicolle* in pig carrion in the state of São Paulo. In Colombia, only adults of *O. discicolle* have been reported; Barreto et al. [7] registered the species in human corpses in Cali; and Wolff et al. [8] in pig carrion in Medellín. In Venezuela, *O. discicolle* was reported breeding on rat carrion in Miranda State [9]. In addition, it has been observed in chicken carrion and carcasses of wild mammal species like opossum, *Didelphis marsupialis* (Velásquez, pers. obs.), and three toed sloth, *Bradypus tridactylus* (Viloria, pers. obs.), as well as associated with amphibian carrion (D. Sánchez, pers. com.).

There is little information regarding the developmental patterns of beetles of forensic importance. Richardson & Goff [10] studied the effects of temperature on the development of *Dermestes maculatus* DeGeer (Coleoptera: Dermestidae). Relating to silphids, there are no papers providing development data forensically useful. Therefore, this study was conducted to determine the effect of temperature on development of the immature stages of *O. discicolle*, in the laboratory under three constant temperatures and under natural conditions.

Methods

Adults of *O. discicolle* were collected from small mammal carcasses and from pitfall traps baited with a piece of chicken meat, in a cloud forest in Altos de Pipe (10°20'N, 66°55'W, 1689 m) Miranda State, Venezuela, from July to November 2005. With specimens obtained, a colony was established in an insectary at the Laboratorio de Biología de Organismos, Centro de Ecología, IVIC. The colony was maintained at 22-24°C, with 70-80% RH and a photoperiod of 12:12 (L:D). The beetles were bred in plastic containers with perforated cover and the bottom was covered with soil (\approx 5 cm) taken from the collection site. They were provided with a piece of chicken muscular tissue (50 g) as food. Water was supplied by soaked cotton pads.

To determine the effects of temperature on the rate of development, 12 pairs of unmated adults (female/male) were removed from the principal colony and placed in plastic jars (11.5 cm diameter x 7.5 cm deep) with perforated cover and the bottom was covered with soil (\approx 5 cm) taken from the collection site. A piece of chicken muscular tissue (\approx 25 g) was provided as food. Water was supplied by soaked cotton pads. The jars were placed into environmental chambers at one of the three constant temperatures of 15, 20, and 28°C, with 70-80% RH and a photoperiod of 12:12 (L:D). The development of *O. discicolle* was also studied under natural conditions, from November 08 to December 09, 2005. The jars were kept outdoors in cages in a shaded cloud forest of Altos de Pipe (10°20'N, 66°55'W, 1689 m), dominated by Apocynaceae (*Aspidosperma fendleri*) and Podocarpaceae (*Podocarpus pittieri*), reaching up to 20-25 m, with many bamboos, mosses and litter. The mean temperature during the development period was 18.5°C and relative humidity was 90%. The averages of minimum and maximum temperatures were 14.4 and 20.0°C, respectively.

For each temperature, 12 pairs (female/male) were used. Females deposit eggs masses of 10 to 30 eggs. When about 100 eggs were deposited, approximately 3 hours after the onset of

oviposition, adults were transferred to a different container. At 24-hour intervals, three of the largest larvae were removed from each jar and examined, until the first larva stopped feeding. Stage duration was determined when the first three individuals in each jar had moulted to the next stage, and it was recorded as an average of 12 jars.

For measurement purposes, removed larvae were killed in 75% ethanol. In order to identify to which larval instar they belonged, measures of pronotal width were made (Velásquez, unpublished data), and total body length was measured to build growth curves. Both measurements were made under a binocular microscope using a vernier MAUF (0.1 mm).

Maximal length means of 36 larvae measured every day were plotted against time for each temperature regime. Then, slopes were compared using homogeneity of slopes model (Statistica 6.0[®]). Normality and homogeneity of variance of data was tested. ANOVA was made to compare total duration of complete development between temperatures. If significant differences were found, post hoc pairwise comparisons were made with Tukey test ($\alpha = 0,05$). We used linear regression (Statistica 6.0[®]) of the developmental rates ($y = 1/\text{developmental time}$) on constant temperature (x), to estimate the lower development threshold temperature (tL). Degree-days (DD) were calculated from the equation $DD = y (t - tL)$, where y is the developmental time (days), t is the rearing temperature ($^{\circ}\text{C}$), and tL is the lower development threshold temperature ($^{\circ}\text{C}$). Degree-days were calculated for larval development and total development, at constant temperatures 15, 20, and 28 $^{\circ}\text{C}$.

Results and Discussion

The means of the maximum length of *O. discicolle* larvae were plotted against time for each temperature regime (Fig. 1). Growth curves slopes were significantly different (homogeneity of slopes model, $F_{7,1216} = 709.47$, $p = 0.00$). This suggests that development rate is different between temperatures. It was noted that slopes increase at higher temperature, and

as temperature decreases, the growth rate also decreases. Figure 1 shows a sigmoid development, for all temperatures. Growth rate decreased when larvae reached third instar. During the active feeding period of the third instar, longitudinal growth was slowed and a widening of the body dominated. Larvae reached their maximum length when they buried.

The mean of the minimum duration of each stage and the total development under rearing temperatures is shown in Table 1. ANOVA showed that the temperature has a significant effect on total duration of development ($F_{3,44} = 242.06$, $p < 0.001$). Total time required to complete development from egg to adult emergence was inversely related to temperature, ranging from 40.00 ± 2.73 days at 15°C to 20.33 ± 0.89 days at 28°C . Developmental time in outdoors natural conditions was not significantly different from that obtained at constant temperature 20°C ($p > 0.05$) (Table I). There were no significant differences between times to begin oviposition (mean \pm SD = 1.58 ± 0.67 , 1.50 ± 0.52 , 1.58 ± 0.51 and 1.51 ± 0.53 days at 15, 20, 28 $^{\circ}\text{C}$ and outdoors, respectively; ANOVA, $F_{3,44} = 0.07$, $p = 0.97$).

Developmental rate increased with temperature; 0.0251, 0.0338 and 0.0493 to 15, 20 and 28 $^{\circ}\text{C}$, respectively ($y = 0.0019 * T - 0.0027$; $R^2 = 0.99$, $p < 0.00$) (Fig. 2). From this, a minimum development threshold (t_0) of 1.5°C , was calculated. Table 2 shows the degree-days calculated above the threshold for each stage of development.

Larval growth and total development time under constant temperature regime were not unexpected. We noted an increased range in duration of developmental stages with lower temperatures. However, all individuals completed development at 15°C . During outdoors development conditions, temperatures fluctuated $\pm 8.9^{\circ}\text{C}$, this variation did not affect the developmental time compared with developmental data obtained at constant temperature 20°C ; although, at outdoors temperatures larvae showed slower growth. Therefore, developmental data obtained from constant rearing temperature for this species could be

applied to fluctuating outdoors temperature conditions as long as mean temperature values were comparable.

There is no previous study exploring the effect of different temperatures on the development of *O. discicolle*. The only record related to the development of *O. discicolle*, is a breeding experiment undertaken at the Museum of Zoology of the University of São Paulo (MZUSP) [11]. However, this trial was conducted for taxonomic purposes, to describe the mature larva, pupa and adult, and did not record the environmental conditions under which it was performed.

Blowfly and fleshfly development data are frequently used for postmortem interval estimation. However, in order to make more precise estimates of time since death, accurate information must be generated for all carrion species. Developmental data generated under natural and controlled laboratory conditions of the carrion beetle *O. discicolle*, constitute baseline data for application to appropriate forensic cases in the Neotropical region.

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Table 1. Minimum duration (days) of developmental stages of *Oxelytrum discicolle*, for each temperature regime.

	Stage duration (mean \pm SD)			
	15°C	20°C	28°C	Outdoor (mean=18.5°C)
Egg	5.16 \pm 0.38	2.91 \pm 0.28	2.91 \pm 0.51	3.08 \pm 0.99
Instar 1	2.58 \pm 0.51	2.00 \pm 0.00	1.00 \pm 0.00	1.91 \pm 0.28
Instar 2	3.83 \pm 1.02	2.00 \pm 0.00	1.41 \pm 0.51	1.91 \pm 0.28
Instar 3	15.91 \pm 1.16	13.25 \pm 1.05	9.00 \pm 0.95	14.25 \pm 1.60
Pupa	12.50 \pm 2.02	9.50 \pm 1.67	6.00 \pm 0.00	10.08 \pm 0.79
Egg to adult	40.00 \pm 2.73	29.67 \pm 1.92	20.33 \pm 0.89	31.25 \pm 0.97

Table 2. Accumulated degree-days (ADD) required to *O. discicolle* reach each stage of development, at three constant temperatures. (ADD calculated on threshold of 1.5 °C).

	ADD		
	15 °C	20 °C	28 °C
Instar 1	69.66	53.84	77.12
Instar 2	104.49	90.84	103.62
Instar 3	156.20	127.84	140.98
Pupa	370.98	372.96	379.48
Adult	539.73	548.71	538.48

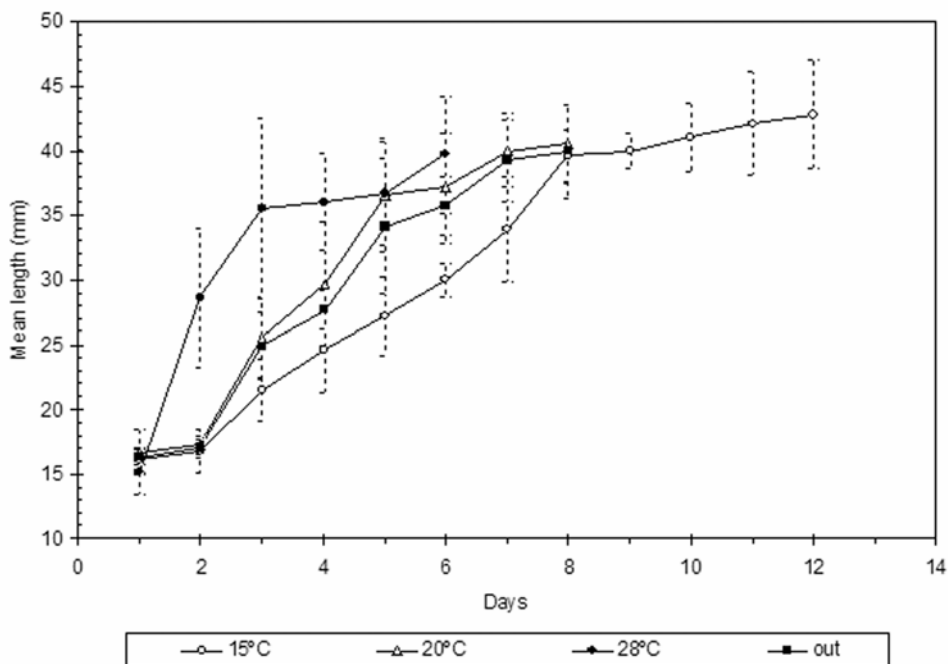


Fig. 1. The mean (\pm SD) length of largest *Oxelytrum discicolle* larvae, measured at 24h intervals for each temperature regime ($n=36$).

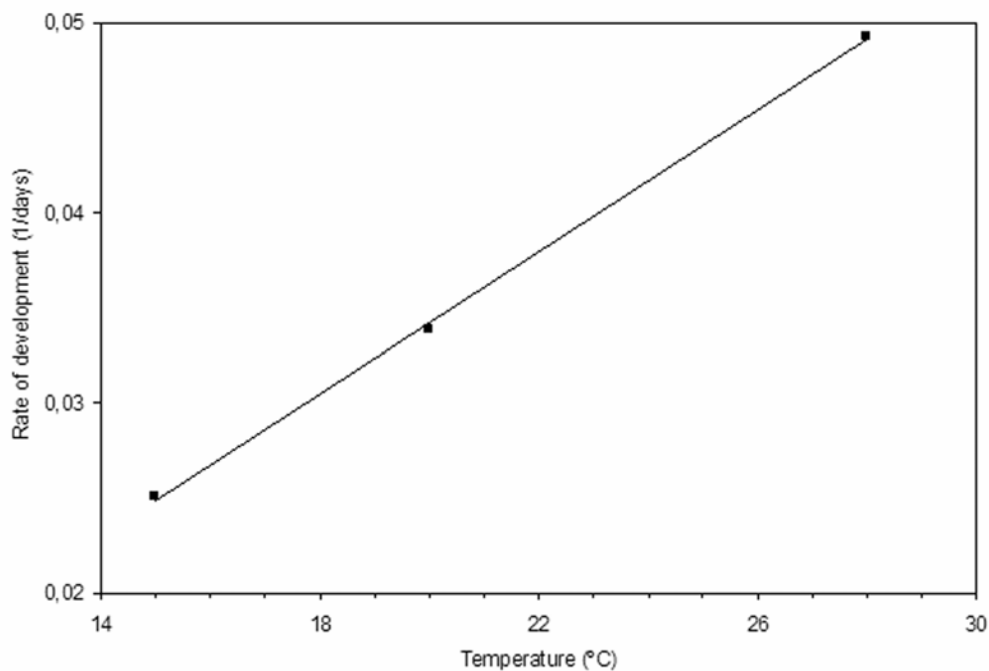


Fig. 2. Relation between the rate of total development (from oviposition to adult emergence) and rearing temperature in *O. discicolle*.