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DEVELOPMENT OF *LUCILIA COERULEIVIRIDIS* (DIPTERA: CALLIPHORIDAE) IN NEW JERSEY, USA

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The developmental stage of insects collected from human or other vertebrate remains can be used to estimate a time of colonization, leading to the possible calculation of a minimum postmortem interval (mPMI) (Catts 1992). *Lucilia coeruleiviridis* (= *Phaenicia*) (Macquart) (Diptera: Calliphoridae) has been found to be the dominant blow fly species in New Jersey, USA during June 2012 comprising 85.3% of adult Calliphoridae collected in beef liver traps (n = 1128) (L.M.W., unpublished data). *Lucilia coeruleiviridis* has also been noted as a common species arriving at pig (*Sus scrofta* L.; Artiodactyla: Suidae) carcasses in Virginia, West Virginia, Florida, and Indiana, USA (Tabor et al. 2004; Joy et al. 2006; Slone & Gruner 2007). However, to the best of our knowledge, there is an absence of development studies conducted on this species. Our study examined the total development time (oviposition to adulthood) of *L. coeruleiviridis* as well as the successful survivorship to adulthood under confined experimental conditions.

A gravid *L. coeruleiviridis* female was captured over a pig carcass on 17 Aug 2012 (N 40° 28' 30.14" -W 74° 26' 24.1"). It was immediately placed into a BugDorm-1 collapsible insect rearing cage (Mega View Science Co., Ltd. Taichung, Taiwan), and placed on a lab bench in a laboratory room set to 24.1 ± 0.4 °C, 14:10 h L:D and ~ 60% RH. The following day, approximately 5 g of fresh beef liver were placed into the BugDorm. All eggs deposited on the beef liver were collected after 5 h. The eggs were then placed onto 5 g of fresh beef liver in a 29 mL translucent plastic SOLO® cup and checked every 6 h for hatching. The resulting larvae were placed onto fresh beef liver at a 1:2 ratio (grams:larvae), and observed twice daily for maturation to third instars.

On 22 Aug 2012, the container was moved to a walk-in incubator (T775A, B, C, D Remote Temperature Controller) that was set to 25 °C (25.5 ± 1.9 °C), 14:10 h L:D and ~60-70% RH. Humidity was checked daily and temperature was recorded in 30-min increments using a HOBO Temperature/Light Data Logger (model UA-002-64K Onset Computer Corp. Cape Cod, Massachusetts). Third instar larvae were partitioned into 2 equal groups of 25 and placed onto 10 g of beef liver and in one of two 56.8 L coolers on a varying substrate. Each cooler contained a 5 cm bottom layer of Kolorscape Washed Play Sand (Oldcastle Lawn Garden, Inc, Atlanta, Georgia). Additionally, cooler one was filled with pine wood shavings until ¾ full, and cooler two was filled with a leaf litter mixture (> 50% local oak leaves) until ¾ full. Each cooler had a wire mesh screen taped over the top to prevent migrating maggots from escaping.

Larvae in the coolers were then checked every 24 h for pupae by sifting the substrate. Observations were originally scheduled to occur every 8 h, but this was found to disturb the larvae (as determined by watching larvae elongate themselves and migrate away from the human disturbance). Each individual pupa was placed into a 30 mL glass vial with a breathable lid and checked every 24 h for emergence. Once an adult was observed the date, time, and sex were recorded, after which the adults were released into a single BugDorm to start a F_1 colony. The colony was supplied with fresh water and pure sugar (Domino® Sugar Premium PureCane Granulated Domino Foods, Inc. Yonkers NY) ad libitum.

Adults were given a paper towel soaked in bovine blood for 5 h/day for 5 days. These steps were taken to ensure that the females had an adequate protein source and oviposition site. On 19 Sep 2012 the colony was moved to a Thermo Scientific Precision Incubator set to 27.0 °C (27.5 ± 0.7 °C), and for the next 20 days, 5-55 g of fresh beef liver were placed into the colony for 5 h. Increments of beef liver were increased by 5 g every third day. After 20 days, approximately 144 g of beef liver were added to the BugDorm container. Three egg clutches were obtained during this time. Each was placed on fresh beef liver (~10 g) in an open quart container and observed every 12 h for hatching for a total of 48 h. In this trial, none of the eggs successfully hatched.

Of the 50 larvae collected from the initial egg clutch, 46 pupated. Of these, 38 emerged from their puparia; 35 (70%) became viable adults. Two adults died shortly after emergence, and 1 fly died during emergence (sex could not be determined). For the 37 pupae that emerged, the sex ratio was approximately 1:1 (19 M:18 F). Development was analyzed using accumulated degree hours (ADH) with a commonly accepted minimum base temperature for this species of 10

°C (B-10) (Deonier 1940; Wall 1992). The overall amount of time needed to reach adulthood from oviposition ranged from 17-23 days (6445.74 – 8588.22 ADH-B10), and averaged 18.7 ± 1.8 days (7069.55 ± 682.14 ADH B-10). The total length of time from oviposition to pupation ranged from 7-13 days (mean = 10.4 ± 1.6 days; Fig. 1), while the amount of time spent as pupae ranged from 6-10 days (mean = 8.3 ± 1.1 days; Fig. 2). There was a significant difference (t = 3.51; df = 33.98; P = 0.001) in the average length of time for total development based on sex, with females (19.7 ± 1.7 days) taking longer to develop than males (17.9 ± 1.5 days). All adults were identified morphologically using a published key (Whitworth 2006) and specimens were sent to the author of that key for verification.

Lucilia coeruleiviridis are challenging to maintain in the laboratory, which may explain why no previous data on their development have been published. Along with *L. coeruleiviridis*, the most common species found in baited beef liver traps and at pig carcasses in New Jersey are *Lucilia sericata* (Meigen) and *Phormia regina* (Meigen) (L. M. W., unpublished data). Knowing their development times has important forensic implications with regards to estimating a time of colonization and inferring a possible mPMI. When reared at 25.0 ± 0.5 °C *L. sericata* were found to have total development times ranging from 329 to 505.5 h, with an average of 448.9 ± 38.8 h with conditions similar to the present study (Tarone & Foran 2006). *Lucilia sericata* was also found to spend an average of 208.3 ± 58.9 h in the pupal phase. In contrast, when *P. regina* were reared at 26 °C, they took 312.0 ± 26.4 h (13.0 ± 1.1 days) to complete development (Nabity et al. 2006). In the present study, the pupal phase of *L. coeruleiviridis* lasted an average of 199.2 ± 26.6 h and total development was completed in 448.8 ± 43.2 h. Thus, perhaps unsurprisingly, the development times of *L. coeruleiviridis* are more similar to those of their congener *L. sericata*.

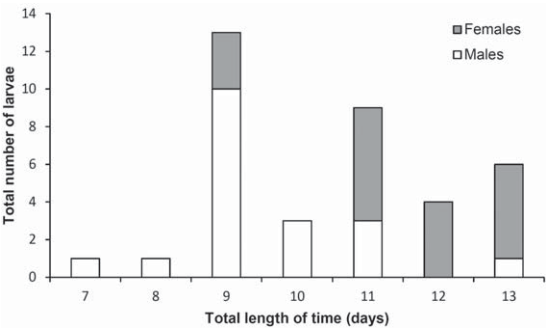

Fig. 1. Total length of time (days) from oviposition to pupation at 25.5 ± 1.9 °C, 14:10 h L:D and ~60-70% RH based on sex.

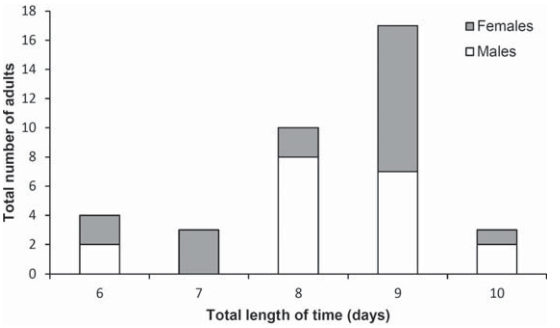

Fig. 2. Total length of time (days) spent in the pupal phase at 25.5 ± 1.9 °C, 14:10 h L:D and ~60-70% RH based on sex.

These data can be used as a guideline for rearing procedures for future studies pertaining to this species. Although egg eclosion did not occur in the present study, eggs successfully eclosed when a clutch was placed on an open 9 cm petri dish, suggesting that airflow may influence this process. The small number (n = 37) of successfully hatched adults in this experiment provides baseline data for the development of *L. coeruleiviridis*. However, as our results stem from one wild caught female, more work focusing on their development using different populations is needed to determine how general these findings are for this forensically important species.

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Summary

Blow fly development is frequently used to help determine a time of colonization, which can be used to infer a minimum postmortem interval (mPMI) in death investigations. Fifty larvae of the blow fly *Lucilia coeruleiviridis* were studied at 25 °C, 14:10 h L:D and ~60-70% RH. Daily observations were made, and total development times were determined. To our knowledge, this is the first study to record development times for this species.

Key Words: forensic entomology, green bottle fly, mPMI, rearing procedure

Resumen

Se utiliza con frecuencia como ayuda el desarrollo de las moscas califóridas para determinar el tiempo de colonización, que puede ser usado

para inferir el intervalo mínimo de postmortem (ImPM) en investigaciones de muerte. Se estudió cincuenta larvas de la mosca califórida, *Lucilia coeruleiviridis* a 25 °C, 14:10 horas L: O y ~60-70% humedad relativa. Se hicieron observaciones diarias y se determinó el tiempo total de desarrollo. En base a nuestro conocimiento, este es el primer estudio para registrar el tiempo de desarrollo de esta especie.

Palabras Clave: entomología forense, mosca botella verde, ImPM, procedimiento de criar

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