

**Laboratory Colonization of the Blow Flies, *Chrysomya*
Megacephala (Diptera: Calliphoridae) and *Chrysomya rufifacies*
(Diptera: Calliphoridae)**

Author(s): Sonja Lise Swiger, Jerome A. Hogsette, and Jerry F. Butler

Source: Journal of Economic Entomology, 107(5):1780-1784. 2014.

Published By: Entomological Society of America

URL: <http://www.bioone.org/doi/full/10.1603/EC14146>

BioOne (www.bioone.org) is a nonprofit, online aggregation of core research in the biological, ecological, and environmental sciences. BioOne provides a sustainable online platform for over 170 journals and books published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Web site, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/page/terms_of_use.

Usage of BioOne content is strictly limited to personal, educational, and non-commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

Laboratory Colonization of the Blow Flies, *Chrysomya megacephala* (Diptera: Calliphoridae) and *Chrysomya rufifacies* (Diptera: Calliphoridae)

SONJA LISE SWIGER,^{1,2,3} JEROME A. HOGSETTE,⁴ AND JERRY F. BUTLER¹

J. Econ. Entomol. 107(5): 1780–1784 (2014); DOI: <http://dx.doi.org/10.1603/EC14146>

ABSTRACT *Chrysomya megacephala* (F.) and *Chrysomya rufifacies* (Macquart) were colonized so that larval growth rates could be compared. Colonies were also established to provide insight into the protein needs of adult *C. rufifacies* and developmental rates of the ensuing larvae. The *C. megacephala* and *C. rufifacies* laboratory colonies were reared for five and six generations, respectively, at 28°C. *C. megacephala* developmental mean rate from egg to adult was 20.4 ± 0.38 d. First-instar larvae emerge in 1.4 ± 0.24 d, second-instar larvae develop in 2.6 ± 0.38 d and third instars occur at 6.3 ± 0.72 d. Development from egg to pupation occurred in 12 ± 1.10 d. *C. rufifacies* developed at a mean rate of 16.2 ± 0.78 d from egg to adult emergence. Each stage occurred in succession from first-instar larvae 1.1 ± 0.25 d, second-instar larvae developed 2.3 ± 0.25 d later, and the third-instar larvae developed 5.7 ± 0.41 d later. The larvae pupated 10.0 ± 0.57 d after oviposition. Both of these flies can be collected in the wild and easily colonized using conditioned chicken as an oviposition and larval medium. *C. megacephala* apparently prefers a lower development and maintenance temperature than *C. rufifacies*, as evidenced by the high pupal mortality. Laboratory-reared *C. rufifacies* benefited from bloodmeal as a protein supplement to enhance egg production. *C. rufifacies* larvae were not observed preying on each other and additional larval species were not provided to serve as prey.

KEY WORDS temperature, bloodmeal, protein source, forensic entomology, chicken thigh

Forensic entomologists often use laboratory colonies of insects when conducting research experiments. Developing and maintaining a colony of flies has many advantages, including control over the life stage, sex, age, number, and quality of insects used, and manipulation of some environmental conditions while holding other conditions constant.

Two blow fly species were chosen for laboratory colonization: *Chrysomya rufifacies* (Macquart) and *Chrysomya megacephala* (F.). *C. rufifacies* and *C. megacephala* are invasive species first reported in the United States in 1982 (Richard and Ahrens 1983) and 1987 (Greenberg 1988), respectively, that have dispersed throughout the continental United States. Both species are important where they occur for decomposition because of their ability to detect and use a carcass (Gruner et al. 2007, Swiger et al. 2014). *C. rufifacies*, a primary carcass colonizer, oviposits only after other calliphorids at the carcass have oviposited. Larvae of *C. rufifacies* become facultative predators as late second

instars and use the larvae of the other calliphorids for food (Goff et al. 1988, Goodbrod and Goff 1990, Swiger et al. 2014). Because of its presence on carcasses found throughout the state of Florida and the value of this species to forensic crime scene investigators, the objective of our study was to rear *C. rufifacies* and *C. megacephala* in the laboratory to obtain more information on developmental rates and behavior.

Materials and Methods

Colonies of both fly species were started by collecting larvae from a 10.4 kg pig carcass exposed in the Natural Area forest adjacent to the University of Florida, Department of Entomology and Nematology, Gainesville, FL. A piece of 19-gauge multipurpose mesh wire (91 by 154 cm) was fitted over the pig to protect against scavengers. The pig carcass was exposed on 10 May 2007. The next day (11 May), >100 second-instar *C. megacephala* larvae were collected from the carcass and >200 second- and third-instar *C. rufifacies* larvae were subsequently collected on 14 May 2007.

The laboratory colonies were started with the collection of second-instar larvae from the decomposing pig. *C. megacephala* larvae were collected from the mouth and snout of the pig, and *C. rufifacies* were collected from the pig's abdomen 48 and 72 h, respectively, after the pig was placed in the field. Several earlier at-

¹ Department of Entomology and Nematology, University of Florida, P.O. Box 110620, Gainesville FL 32611.

² Present address: Texas A&M AgriLife Extension, 1229 N. U.S. Hwy 281, Stephenville, TX 76401.

³ Corresponding author, e-mail: slswiger@ag.tamu.edu.

⁴ United States Department of Agriculture, Center for Medical, Agricultural and Veterinary Entomology, 1600 SW 23rd Dr., Gainesville, FL 32608.



Fig. 1. A Ziploc container and conditioned chicken thighs used to rear developing *C. megacephala* and *C. rufifacies* larvae from egg to wandering larvae stage. (Online figure in color.)

tempts were made to collect larvae with calf liver and chicken thighs with no success. *C. rufifacies* are observed to be frequent visitors of fresh mammalian carcasses but could not be collected with fresh meats purchased from a local market (Swiger et al. 2014).

All larvae collected from the pig were reared at the U.S. Department of Agriculture (USDA) Center for Medical, Agricultural and Veterinary Entomology in a walk-in growth chamber (2.4 by 2.4 by 2.4 m) initially at 25°C but later increased and maintained at 28°C and 56% relative humidity with constant illumination from an overhead fluorescent light and a floor-mounted UV insect light trap.

Eggs deposited by reared adults were allowed to hatch on, and larvae develop in, conditioned chicken thighs (168.3 ± 5 g each). Conditioned chicken was produced by placing fresh chicken thighs from a local super market in Ziploc plastic containers (28 by 17.7 by 8.5 cm) and leaving them in the growth chamber for ≥2 d with the plastic lids in place (Fig. 1). Ziploc containers of this size were used for all subsequent oviposition, larval development, and pupal eclosion unless otherwise stated.

Ziploc containers in which larvae were developing were placed in Hefty EZ Foil Cake Pans (12 1/4 × 8 1/4 × 1 1/4 inch in depth [31 by 21 by 31 cm]) to contain any wandering late third-instar larvae that escaped from the Ziploc containers. Wandering third-instar larvae were collected with forceps or gloved hands, placed in Ziploc containers containing a 5-cm layer of dry, white, leveling sand (Gravelscape, Sanford, FL) and allowed to pupate. Larvae that did not wander from the chicken were collected with gloved hands before or shortly after pupation and placed in the same containers.

Pupae were removed manually from the sand and groups of 50–100 were placed in squat plastic cups (4 by 11.5 cm diameter). Cups with pupae were placed individually in standard colony cages (38 cm in width by 38

cm in height by 46 cm in length) constructed of aluminum window framing with sheet metal floors. The two sides, back and top, were covered with standard window screen (18 by 16 mesh), and the open end of the cages was secured with 6-inch (15-cm)-wide tubular cotton stockinet (Independent Medical Co-Op. Inc., Ormond Beach, FL). Water, sugar, and powdered milk were provided ad libitum to each cage of insects. Moistened bloodmeal was also provided ad libitum for *C. rufifacies* adults upon emergence to promote oviposition.

Approximately 3–6 d after emergence, *C. megacephala* and *C. rufifacies* adults were given a conditioned chicken thigh in a squat cup to encourage mating and promote oviposition. The chicken and the cup were removed from the cage after 24 h and the chicken, with eggs, was placed in a Ziploc container for eggs to hatch and larvae to develop. Two additional conditioned chicken thighs were added to each Ziploc container for use by the larvae. Each Ziploc container was placed in a Hefty EZ Foil Cake Pan as stated above to contain wandering larvae.

The number of eggs oviposited by 50–100 *C. megacephala* and *C. rufifacies* females, in 24 h was between 200 and 300 per chicken thigh. Larval development was measured for all the generations by selecting 10 larvae daily from each colony and determining the number of larvae present in each instar. Instar was determined by viewing the characteristic posterior spiracles of each larva under a dissecting scope (James 1947). The larvae were then returned to their respective colonies to continue development.

In addition, studies were conducted to determine the eclosion rates of eggs and pupae. Ten freshly laid eggs were placed in each of the four folded, moist paper towel sections (28 by 23 cm) and placed individually in petri dishes (8.8 cm in diameter). Petri dishes were covered and placed in the growth chamber overnight. The next morning, the number of larvae present in



Fig. 2. A single *C. ruffacies* egg mass laid upon a conditioned chicken thigh. (Online figure in color.)

each petri dish was counted and recorded. The eclosion rate was determined by placing 10 pupae in each of the four small Styrofoam cups (6 cm in height by 8.6 cm in diameter) that were then covered with petri dishes. Pupae were held in the growth chamber until adults eclosed (4–7 d). Adults were counted to determine the eclosion rate and female to male sex ratios were recorded after adults were killed by freezing.

Results

The *C. megacephala* adults collected as larvae from the pig carcass emerged 5–9 d after pupation. Mating

began ≈2 d after adult emergence and oviposition occurred at 3–4 d of age. Eggs were deposited in groups and collected groups were found to contain 200–300 eggs each (Fig. 2). Eggs held at 28°C hatched within 1.4 ± 0.24 d (Fig. 3). The egg eclosion rate was 55% (Table 2). *C. megacephala* grew well at densities of 200–300 larvae per two chicken thighs. Second instars were present 4.0 d after hatching, becoming third instars 2.6 ± 0.38 d later (Table 1). Larvae remained in the third stadium for 6.3 ± 0.72 d. *C. megacephala* larvae in the densities mentioned above never consumed more than three chicken thighs. Mean *C. megacephala* adult emergence rate was 37.5% (Table 2).

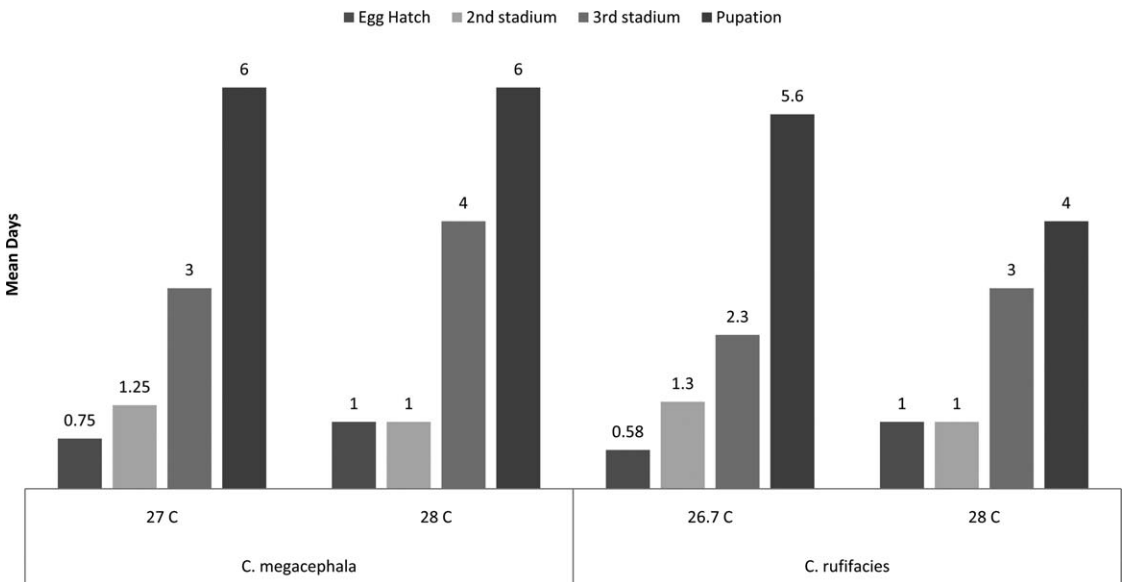


Fig. 3. Mean number of days for colony-reared *C. megacephala* and *C. ruffacies* to develop from egg to pupae at 28°C, compared with 27°C data of Wells and Kurahashi (1994) and 26.7°C of Byrd (1995), respectively.

Table 1. Mean time recorded in days for *C. megacephala* development from egg to adult emergence

Period of development*	F ₂	F ₃	F ₄	F ₅	Mean (± SE)
First stadium	1.0	2.0	1.0	1.5	1.4 (±0.24)
Second stadium	2.0	3.5	2.0	3.0	2.6 (±0.38)
Third stadium	6.0	6.5	4.5	8.0	6.3 (±0.72)
Pupation	11.0	14.5	9.5	13.0	12.0 (±1.10)
Adult emergence	21.0	20.0	19.5	21.0	20.4 (±0.38)

For all stages except adult emergence, values indicate days spent in that stage.
*n = 10.

Females accounted for 35.5% of the adult flies. Five generations of *C. megacephala* were reared. Mean development time from egg to adult was 20.4 ± 0.38 d.

C. rufifacies adults collected as larvae from the pig carcass emerged 4–5 d after pupation. Mating was observed ≈4 d after emergence with oviposition occurring at 6 d of age. One to two egg groups were deposited on a single chicken thigh; each group contained 200–300 eggs. The egg eclosion rate was 68% (Table 2). Within 1 d (1.1 ± 0.25 d) after eggs were deposited, first- and sometimes second-instar larvae were present (Table 3). Third-instar larvae were first observed 4.4 d after egg emergence. The *C. rufifacies* larvae remained in the third stadium for 5.7 ± 0.41 d with pupation occurring 10.0 ± 0.057 d after egg eclosion. All chicken provided was consumed by the larvae throughout their development and sometimes another piece had to be added. Adult emergence rate was 80.0% (Table 2), with females accounting for 78.0% of the adults. The *C. rufifacies* laboratory colony was reared for a total of six generations. Mean development time from egg to adult was 16.2 ± 0.78 d.

Discussion

C. megacephala has been reared in laboratory colonies for research purposes, most notably by Wells and Greenberg (1992a,b) and Wells and Kurahashi (1994). The laboratory colony reported in Wells and Kurahashi (1994), was reared under a photoperiod of 16:8 (L:D) h at 27°C, and larval growth rates were recorded daily. The growth rates of our colony compared well with the data collected by Wells and Kurahashi (1994). Egg hatch was recorded at 0.75 d (18 h) by Wells and Kurahashi (1994), and at 1 d (24 h) in our study. Second-instar larvae were recorded 1.25 d (30 h) after the first instars by Wells and Kurahashi (1994), and 1 d (24 h) after the first instars in the current study. Wells and Kurahashi (1994) observed

Table 2. Eclosion rates of *C. megacephala* and *C. rufifacies* eggs and pupal stages to determine survivability within a laboratory colony

Species	Mean % egg eclosion	Mean % adult eclosion
<i>C. megacephala</i>	55.0 ± 2.38	37.5 ± 1.71
<i>C. rufifacies</i>	68.0 ± 2.06	80.0 ± 0.82

Table 3. Mean time recorded in days for *C. rufifacies* development from egg to adult emergence

Period of development*	F ₂	F ₃	F ₄	F ₅	F ₆	Mean (± SE)
First stadium	1.0	0.5	2.0	1.0	1.0	1.1 (±0.25)
Second stadium	1.5	2.0	3.0	2.5	2.5	2.3 (±0.25)
Third stadium	5.0	6.0	6.5	6.5	4.5	5.7 (±0.41)
Pupation	10.0	11.5	10.0	10.5	8.0	10.0 (±0.57)
Adult emergence	14.5	18.0	14.5	18.0	16.0	16.2 (±0.78)

For all stages except adult emergence, values indicate days spent in that stage.
*n = 10.

third-instar larvae 3 d (72 h) after the second-instar larvae followed by pupae 6 d (144 h) later. In our study, *C. megacephala* developed into third-instar larvae 4 d (96 h) after second instars appeared and began to pupate 6 d (144 h) later.

Our *C. megacephala* larvae developed under the set laboratory conditions needed to maintain the *C. rufifacies* colony but the 28°C temperature in our growth chamber and the rearing medium of conditioned chicken thighs, may have been more than optimal. Wells and Kurahashi (1994) indicate that 27°C was the temperature used by other researchers for rearing *C. megacephala* and previous colonies were reared on beef or pork liver (Wells and Greenberg 1992b, Wells and Kurahashi 1994). Although our larvae developed normally at 28°C, there was high pupal mortality (Table 1) and a pupal eclosion rate of only 37.5%.

C. rufifacies adults were found to be very sensitive to ambient temperature variations of the growth chamber. *C. rufifacies* is considered a warm weather species and the larvae prefer high temperatures (Williams and Richardson 1984). The temperature of the growth chamber during the development of the wild-collected larvae, adult F₂, and larvae from F₂ adults was 28°C because the exhaust fan in the growth chamber was not operating properly. At this temperature, the adults oviposited several times and did not suffer from premature mortality. When the growth chamber fan was repaired, the temperature within was 25°C, which appeared to be unsuitable for *C. rufifacies*. Mating occurred but oviposition did not. Two cages of adults were present at this temperature, and all the adults in the second cage died prematurely. Even with the increased ambient temperature in the rearing chamber, from 25 to 28°C, only two batches of eggs were produced by the adult F₃ *C. rufifacies* adults over a 2-wk period. When moistened bloodmeal was provided to the remaining 2-wk-old *C. rufifacies* adults, oviposition then occurred and eggs were produced in vast numbers for several days. Moistened bloodmeal was provided thereafter to the adult *C. rufifacies* every day after emergence to promote oviposition. In hindsight, it appears that the adult *C. rufifacies* were deprived of necessary nutrients before the addition of moistened bloodmeal and the temperature might not have been as big of a concern but the chamber remained at 28°C for the remainder of the study period. The temperature variations did not impact the developing larvae, and therefore no data were omitted.

Literature on *C. rufifacies* growth rates is scant. Our colony data were compared with those of Byrd (1995) for *C. rufifacies* reared at 26.7°C. Overall development for *C. rufifacies* recorded by Byrd was a day longer: egg hatch 0.58 d (14 h) after oviposition, second-instar larvae 1.3 d (32 h) later, third-instar larvae 2.3 d (56 h) after the second instars and pupation 5.6 d (134 h) after the third instars. Our colony developed at an average of 1 d (24 h) for egg hatch after oviposition, 1 d (24 h) later second-instar larvae were present, followed by third-instar larvae 3 d (72 h) later and then pupation occurred 4 d (96 h) after the third instars. Under field conditions, we found pupation to occur 12 d after first-instar larvae were collected at an average ambient temperature of 25.56°C during August (Swiger et al. 2014).

The competitive nature of *C. rufifacies* is seen in the short time to pupation, early adult emergence, despite delayed oviposition. *C. rufifacies* larvae are known to be facultative predators of larvae of other fly species and previous attempts to colonize *C. rufifacies* without the inclusion of larvae of other flies for them to feed on have been unsuccessful. However, our colony was reared on conditioned chicken alone, with a protein source (bloodmeal) added to the adult diet for egg production.

C. rufifacies is one of the first flies to arrive at carrion in the wild but it does not oviposit until 24 h after the death of the host. The adults have been observed resting on nearby trees and vines during those 24 h. It now seems possible that the adult flies are arriving early at a carcass to feed on nutrients required to complete egg development before the subsequent oviposition on the carcass (Swiger et al. 2014). The discovery that *C. rufifacies* colony adults needed supplemental nutrients to produce and lay eggs when reared on conditioned chicken thighs provides insight into the adults' nutritional needs. The necessity for supplemental nutrients in the colony diet indicates that the adult females were not consuming enough of the required nutrients from the chicken thighs for oviposition to occur. The chicken provided larvae with sufficient nutrients for development but did not supply the adults with the nutrients required for egg development. Bloodmeal, noted for its high (50%) protein content, provided nutrients not present in the chicken thighs, which were needed for oviposition to occur. Other dipteran adults, e.g., *Hydrotaea aenescens* (Wiedemann), need dietary supplements, namely, protein sources, to oviposit and develop properly (Hogsette and Washington 1995), especially when colonized in the laboratory.

So, in summary, both *C. megacephala* and *C. rufifacies* can be collected in the wild and easily colonized using conditioned chicken as an oviposition and larval medium. *C. rufifacies* benefits from bloodmeal as an additional protein supplement to enhance egg production. Development times from egg to adult for *C. megacephala* and *C. rufifacies* were \approx 20 and 16 d, respectively, under the conditions of this study.

Acknowledgments

S.L.S. gives heartfelt thanks to all of the USDA fly colony technicians who suffered, wretched, and griped through the stench produced during this study, and to my family and friends, who allowed me to wear my "conditioned" clothes for the rest of the day and through the supper hour!

References Cited

- Byrd, J. H. 1995. The effects of temperature on flies of forensic importance in Florida. M.S. Thesis, University of Florida, Gainesville.
- Goff, M. L., A. I. Omori, and K. Gunatilake. 1988. Estimation of postmortem interval by arthropod succession: Three case studies from the Hawaiian Islands. *Am. J. Forensic Med. Pathol.* 9: 220–225.
- Goodbrod, J. R., and M. L. Goff. 1990. Effects of larval population density on rates of development and interactions between two species of *Chrysomya* (Diptera: Calliphoridae) in laboratory culture. *J. Med. Entomol.* 27: 338–343.
- Greenberg, 1988. *Chrysomya megacephala* (F.) (Diptera: Calliphoridae) collected in North America and notes on *Chrysomya* species present in the New World. *J. Med. Entomol.* 25: 199–200.
- Gruner, S. V., D. H. Slone, and J. L. Capinera. 2007. Forensically important Calliphoridae (Diptera) associated with pig carrion in rural north-central Florida. *J. Med. Entomol.* 44: 509–515.
- Hogsette, J. A., and F. Washington. 1995. Quantitative mass production of *Hydrotaea aenescens* (Diptera: Muscidae). *J. Econ. Entomol.* 88: 1238–1242.
- James, M. T. 1947. The flies that cause myiasis in man. Government Printing Office, WA, No. 631.
- Richard, R. D., and E. H. Ahrens. 1983. New distribution record for the recently introduced blow fly *Chrysomya rufifacies* (Macquart) in North America. *Southwest Entomol.* 8: 216–218.
- Swiger, S. L., J. A. Hogsette, and J. F. Butler. 2014. Larval distribution and behavior of *Chrysomya rufifacies* (Macquart) (Diptera: Calliphoridae) relative to other species on Florida black bear (Carnivora: Ursidae) decomposing carcasses. *Neotrop. Entomol.* 43: 21–26.
- Wells, J. D., and B. Greenberg. 1992a. Interaction between *Chrysomya rufifacies* and *Cochliomyia macellaria* (Diptera: Calliphoridae): the possible consequences of an invasion. *Bull. Entomol. Res.* 82: 133–137.
- Wells, J. D., and B. Greenberg. 1992b. Laboratory interaction between introduced *Chrysomya rufifacies* and native *Cochliomyia macellaria* (Diptera: Calliphoridae). *Environ. Entomol.* 21: 640–645.
- Wells, J. D., and H. Kurahashi. 1994. *Chrysomya megacephala* (Fabricius) (Diptera: Calliphoridae) development: Rate, variation and the implications for forensic entomology. *Jpn. J. Sanit. Zool.* 45: 303–309.
- Williams, H., and A.M.M. Richardson. 1984. Growth energetics in relation to temperature for larvae of four species of necrophagous flies (Diptera: Calliphoridae). *Aust. J. Ecol.* 9: 141–152.

Received 7 April 2014; accepted 6 August 2014.