Effects of Temperature and Tissue Type on the Development of *Cochliomyia macellaria* (Diptera: Calliphoridae)

STACY A. BOATRIGHT[1] AND JEFFERY K. TOMBERLIN[1,2]

J. Med. Entomol. 47(5): 917–923 (2010); DOI: 10.1603/ME09206

ABSTRACT The secondary screwworm, *Cochliomyia macellaria* (Fabricius), was reared on either equine gluteus muscle or porcine loin muscle at 20.8°C, 24.3°C, and 28.2°C. *C. macellaria* needed ≈35% more time to complete development when reared at 20.8 than 28.2°C. Furthermore, larval growth and weight over time did not differ between larvae reared on equine versus porcine muscle. This study is the second in the United States to examine the development of *C. macellaria* and is the first to examine development of this species on muscle tissue from different vertebrate species. These data could provide significant information regarding time of colonization, including myiasis and neglect cases involving humans and animals. Furthermore, these results in comparison with the only other data set available for this species in North America indicate a fair amount of phenotypic variability as it relates to geographic location, suggesting caution should be taken when applying these data to forensic cases outside the region where this study was conducted.

KEY WORDS secondary screwworm, forensic entomology, forensic veterinary medicine, myiasis

Forensic entomology is the utilization of insects and other arthropods as evidence in both civil and criminal investigations (Williams and Villet 2006). The broad scope of this field can be broken down into three subdivisions, which are urban, stored-product, and medicolegal entomology (Archer 2007). Medicolegal entomology relates to necrophagous arthropods colonizing and feeding on living and necrotic human and animal tissue. Insects found on vertebrate remains may be used to estimate the period of insect activity or minimum postmortem interval (Amendt et al. 2007).

Entomologists also are asked to analyze insect evidence collected from living people and animals. Confirming myiasis, the infestation and subsequent feeding upon living tissue, organs, or bodily fluids of a vertebrate host by dipteran larvae can be used as evidence in cases of suspected neglect or abuse (Anderson and Huitson 2004). Myiasis typically occurs in elderly or very young people, who are unable to care for themselves, as well as in pets and livestock with open wounds or fecal matter present on their bodies for an extended period of time (Anderson and Huitson 2004).

Forensic entomologists depend on growth data from laboratory studies to estimate the period of insect activity for blow flies (Diptera: Calliphoridae) collected from decedents (Tarone and Foran 2006) as well as the living (Anderson and Huitson 2004). Although similar development times have been documented in pre-existing data sets for a variety of blow fly species, some variations can be found within species (Tarone and Foran 2006, Gallagher et al. 2010). Variation could be the result of a number of factors, such as experimental design, environment, and genetic variation. Tarone and Foran (2006) determined that environment conditions greatly influenced the development of the blow fly *Lucilia sericata* (Meigen) (Diptera: Calliphoridae). Their findings demonstrate the need for multiple development data sets for each species of blow fly species across geographic regions, as well as for a standardization of laboratory-rearing techniques.

Most development data used by forensic entomologists are obtained from studies examining the growth of larvae-fed beef liver (Clark et al. 2006). However, it has been demonstrated that the host species on which the larvae feed may significantly alter larval growth rate. For example, development of *L. sericata*-fed lung, liver, and heart, from both cows and swine, was compared (Clark et al. 2006). It was determined that larvae grew significantly faster on swine than on cow. Furthermore, development varied when reared on lung and heart compared with liver of the same animal. For forensic entomologists, such results emphasize the need for development data sets across tissue types as well as across species. The importance of recording the location from which blow fly larvae are removed from a corpse at a body recovery scene was also demonstrated (Clark et al. 2006). Such information can allow for refined estimates of the period of insect activity.

[1] Department of Entomology, Texas A&M University, College Station, TX 77843.

[2] Corresponding author: 2475 TAMU, Department of Entomology, Texas A&M University, College Station, TX 77843 (e-mail: jktomberlin@ag.tamu.edu).

Cochliomyia macellaria (Fabricius), the secondary screwworm (Diptera: Calliphoridae), is a facultative parasite. Unlike the primary screwworm, *Cochliomyia hominivorax* (Coquerel) (Diptera: Calliphoridae), larvae of *C. macellaria* do not feed on living tissue; rather, they colonize a wound and feed on necrotic tissue (Harrison and Pearson 1968). *C. macellaria* is one of the most common colonizers of vertebrate remains in the southern United States, thus making it a fly of great forensic importance (Hall 1948). The secondary screwworm is of particular importance to the livestock industry because of large economic losses resulting from pathogen transmission and myiasis (Anderson and Huitson 2004). The recent increase in the number of criminal and civil investigations in the United States where forensic entomologists are asked to analyze evidence further demonstrates the need for more development data sets to enhance the accuracy of estimating the period of insect activity (Grassberger and Reiter 2001). Currently, the only available development data for *C. macellaria* from the United States are for specimens reared on lean pork in Florida (Byrd and Butler 1996).

The primary objective of this study was to examine the development of *C. macellaria* on equine and porcine striated muscle tissue at different temperatures. We hypothesized that the development rates for *C. macellaria* would not differ across tissue types. Furthermore, we hypothesized that *C. macellaria* development would not differ across the temperatures investigated.

Materials and Methods

Adult Fly Colony. *C. macellaria* used in this experiment originated from larvae collected from decomposing animal carcasses in the vicinity of College Station, TX, from June through August 2008. Collected larvae were fed fresh beef liver in a 1-liter plastic container and housed in a 136LLVL Percival (Percival Scientific, Perry, IA) growth chamber at ≈27°C with 14:10 (L:D) h and 75–80% RH (modified from Byrd and Butler 1996, Nabity et al. 2006). Emergent adult flies were placed in nylon mesh 30 × 30 × 30-cm Bioquip cages and provided a 50:50 sugar:powdered milk mixture and fresh water ad libitum (Byrd and Butler 1996).

Tissue Source. Tissue samples from three equines, *Equus ferus* Boddaert, and three porcines, *Sus scrofa* L., were used in this development study. Equine gluteus muscle was supplied by the Texas Veterinary Medical Diagnostic Laboratory (Texas A&M University, College Station, TX). Tissue acquisition protocols were approved by the Animal Welfare Assurance Program, Texas A&M University. Equines used were free of barbiturates or other materials used for euthanization. Approximately 4 kg of tissue was collected from each of the three equines. Lean boneless pork chops (i.e., loin muscle) were purchased from a local grocery. Varied packets of boneless pork chops were purchased to increase the likelihood of the tissue resulting from different animals. Equine and porcine tissue samples were partitioned into 200-g samples and placed into separate Ziploc sandwich bags, labeled, assigned a temperature treatment, and stored in a −20°C freezer until use.

Experiment Design. The experimental design was adapted from Byrd and Butler (1996) and Nabity et al. (2006). Development time of *C. macellaria* on equine and porcine muscle tissue in 136LLVL Percival upright incubators set at 20.8 ± 1.3°C, 24.3 ± 0.7°C, and 28.2 ± 1.1°C was recorded. An Onset HOBO U12–006 data logger with an Onset TMC6-HD air, water, and soil temperature sensor (Onset, Pocasset, MA) was placed on each shelf in each of the growth chambers, and temperature was recorded hourly throughout the duration of the study.

Equine and pork tissues were removed from the freezer, and 200-g samples were placed individually in 1.10-liter styrene mosquito-breeding containers (Bio-Quip Products, Rancho Dominguez, CA). These were held individually in opaque Sterilite shoebox containers (Sterilite, Townsend, WI), 33 (L) × 19 (W) × 11 (D) cm, layered with 500 ml of sand (Quikrete Premium Play Sand, Atlanta, GA) to prevent wandering larvae from escaping. The containers were not covered and were placed in a Latin square design with three samples of each tissue type in each growth chamber. Tissue samples were inoculated with eggs ≈24 h after placement in the growth chambers. Fly colonies were monitored hourly for oviposition. Eggs <1 h old were mixed in deionized water and weighed gravimetrically, and 400 egg aliquots were placed on each tissue sample, using a fine-tip artist paint brush. Only eggs from 7- to 10-d-old F_1 generation flies were used in this study.

Life History. Eggs on each tissue sample were checked hourly for larval eclosion. Once initial eclosion occurred for eggs on a tissue sample, observations were made every 12 h. During each observation, three larvae were subsampled from the respective container. Each larva sampled was parboiled in water, and then weight, length, instar, and time of observation were recorded. Weights were measured using an Adventure-Pro AV64 Ohaus scale (Ohaus, Pine Brook, NJ). Lengths of larvae were recorded using a stage scale and a Meiji Techno EMZ-8TR microscope (Meiji Techno America, Santa Clara, CA). These observations continued until all larvae had either died or pupated.

Development time of pupae was recorded for the initial 30 larvae to pupate. Pupae were placed individually in 35-ml Jetware (Jetware, Hatfield, PA) plastic medicine cups containing ≈10 ml of sand (Quikrete Premium Play Sand, Atlanta, GA), capped with breathable lids, labeled, returned to the appropriate growth chamber, and monitored every 12 h for adult emergence. Time to adult emergence and sex were recorded for each individual. Sex of each fly was recorded after death. Adults that died during eclosion were not included in adult longevity analysis. Because of unequal subsample sizes per sample, development data were not analyzed by sex; however, overall sex ratio was determined. Adults were monitored every

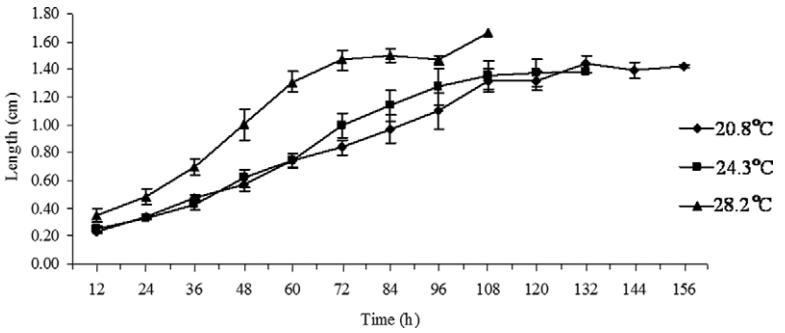

Fig. 1. *C. macellaria* larval weight ($n = 3$) ± SE developing at three temperatures over time.

12 h for mortality. Adult flies were provided with 0.20 ml of distilled water via a 1-ml Kendall Monoject SoftPack insulin syringe (Kendall, Mansfield, MA) inserted through the lid every 24 h. Development and longevity times were converted to degree days with the base temperature set at 10°C. Representative samples of the adult specimens obtained from this study were deposited in the Texas A&M University Insect Collection.

Statistical Analysis. All statistical analyses were performed using SPSS (SPSS 2005). Analysis of covariance (ANCOVA) was used to compare weight and length (dependent variables) data for flies reared on each tissue type across temperatures (independent variables) over time (covariate). Because weight and length data did not meet basic assumptions for parametric analysis, they were ranked before analysis. Development times for stage of development were converted to accumulated degree days and analyzed with a two-way analysis of variance, with temperature and tissue as main effects and samples nested within temperature. Fisher least significant difference test was performed to separate means following a significant F test ($P < 0.05$).

Results

Nontransformed mean length and weight data over time for larvae reared on equine and porcine tissue are presented in Figs. 1 and 2, respectively. Based on our analyses, we failed to reject our null hypothesis. Larval development did not differ across tissue treatments. Larval weight did not significantly differ between tissue types at 20.8°C ($F = 0.157$; df = 1, 65; $P = 0.693$), 24.3°C ($F = 1.005$; df = 1, 54; $P = 0.321$), or 28.2°C ($F = 1.380$; df = 1, 41; $P = 0.247$) (Fig. 3). Hours elapsed significantly predicted larval weight at 20.8°C (slope = 0.993; $t = 0.993$; $P < 0.0001$), 24.3°C (slope = 1.214; $t = 12.224$; $P < 0.0001$), and 28.2°C (slope = 1.708; $t = 12.805$; $P < 0.0001$). Hours elapsed also explained a significant proportion of variance in ranked larval weight for 20.8°C ($R^2 = 0.875$; df = 3, 65; $P < 0.0001$), 24.3°C ($R^2 = 0.863$; df = 3, 54; $P < 0.0001$), and 28.2°C ($R^2 = 0.880$; df = 3, 41; $P < 0.0001$). Hours elapsed significantly predicted larval length at 20.8°C (slope = 1.020; $t = 14.529$; $P < 0.0001$), 24.3°C (slope = 1.225; $t = 11.808$; $P < 0.0001$), and 28.2°C (slope = 1.735; $t = 10.990$; $P < 0.0001$) (Fig. 4). Hours elapsed also explained a significant proportion of variance in ranked larval length for 20.8°C ($R^2 = 0.868$; df = 3, 65; $P < 0.0001$), 24.3°C ($R^2 = 0.850$; df = 3, 54; $P < 0.0001$), and 28.2°C ($R^2 = 0.838$; df = 3, 41; $P < 0.0001$).

Intercept for larval length by hour did not significantly differ between tissue types at 20.8°C ($F = 0.505$; df = 1, 65; $P = 0.480$), 24.3°C ($F = 0.742$; df = 1, 54; $P = 0.393$), or 28.2°C ($F = 0.912$; df = 1, 41; $P = 0.345$) (Fig. 4). Therefore, tissue was removed from the analysis, and ranked mean data for weight and length were analyzed with an ANCOVA, with temperature as the independent variable and hour as the covariate. However, temperature and hour interacted for length ($F = 16.03$; df = 2, 166; $P < 0.0001$) and weight ($F = 16.19$;

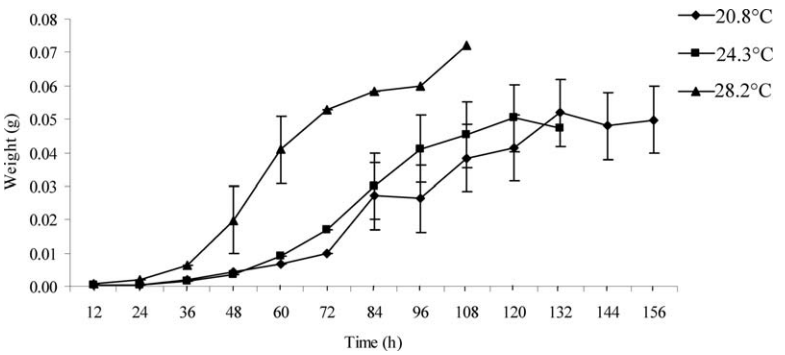

Fig. 2. *C. macellaria* larval length ($n = 3$) ± SE developing at three temperatures over time.

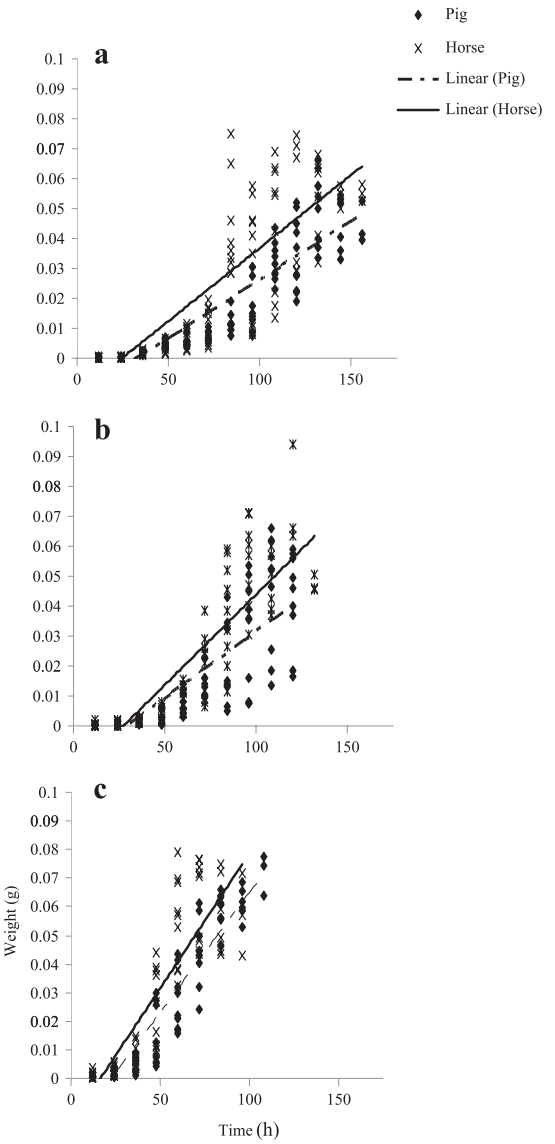

Fig. 3. Ranked weight (g) data using the calculated *x*-intercept and ANCOVA results for larvae reared at (a) 20.8°C, (b) 24.3°C, and (c) 28.2°C.

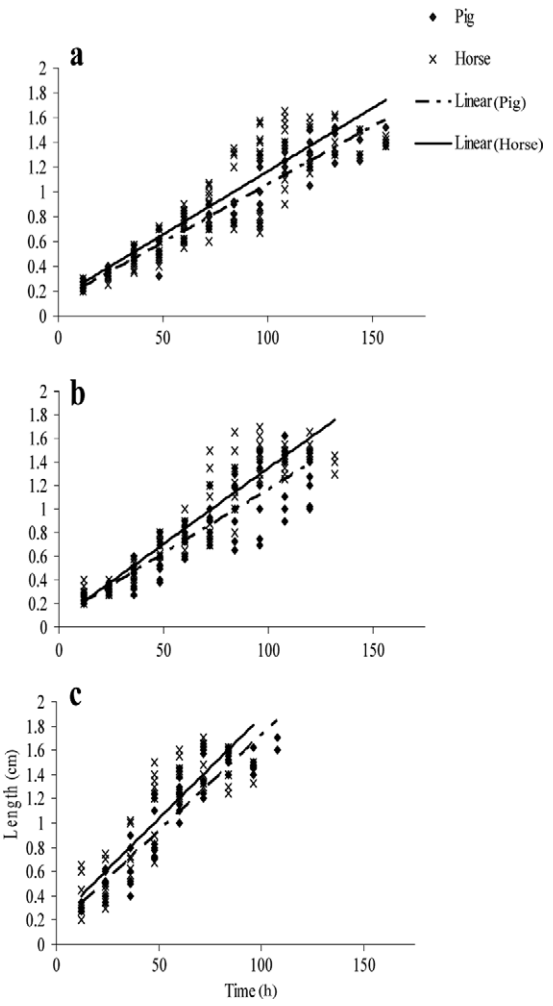

Fig. 4. Ranked length (cm) data using the calculated *y*-intercept and ANCOVA results for larvae reared at (a) 20.8°C, (b) 24.3°C, and (c) 28.2°C.

df = 2, 166; $P < 0.0001$). Therefore, the results of the ANCOVA could not be used.

Degree day data for *C. macellaria* reared on equine and porcine tissue are presented in Table 1 using a base temperature of 10°C. Based on the analysis of variance results, we failed to reject our null hypothesis. Degree days needed for adults reared on porcine and equine muscle were not significantly different ($F = 1.540$; df = 1, 2.5; $P = 0.318$) (Table 1), indicating no tissue effect. However, we did reject our null hypothesis that *C. macellaria* development would not significantly differ across the temperatures investigated. Degree days by instar did in fact differ significantly ($P < 0.05$). An interaction between temperature and instar was also determined ($F = 21.636$; df = 4, 4; $P = 0.0006$) in regard to degree days needed to complete each stage of development. Flies reared at 28.2°C needed ≈31 and 21% less degree days to complete development than those reared at 20.8°C and 24.3°C, respectively. Sex ratios were similar (Table 2) across tissue and temperature. Although not significant, adult longevity (Table 2) was similar across tissue treatments, with those reared at cooler temperatures living longer.

Discussion

Byrd and Butler (1996) assessed the development of *C. macellaria* on porcine tissue across five temperature regimes. Their study included temperatures of 21.1°C and 26.7°C, which should have produced mean development stage values comparable to those recorded at 20.8°C and 28.2°C in our study. However, flies in our study required more degree days to complete each

Table 1. Minimum degree days and accumulated degree days for development stages of *Cochliomyia macellaria* ($n = 3$) on equine and porcine muscle tissue at three temperatures

Temperature (°C)	Tissue		Egg ± SE (DD)	First instar ± SE (DD)	Second instar ± SE (DD)	Third instar ± SE (DD)	Prepupal ± SE (DD)	Pupal ± SE (DD)
20.8	Equine		8.32 ± 0.39	10.90 ± 0.00	30.87 ± 3.64	59.92 ± 6.29	8.60 ± 0.55	69.89 ± 3.12
		Mean ADD		19.22	50.09	110.01	118.61	188.50
	Porcine		8.02 ± 0.31	10.90 ± 0.00	30.87 ± 3.64	65.37 ± 3.15	7.15 ± 0.60	66.17 ± 0.49
		Mean ADD		18.92	49.79	115.16	122.31	188.48
24.3	Equine		10.82 ± 0.39	14.18 ± 0.00	37.80 ± 2.36	66.15 ± 6.25	9.57 ± 1.83[a]	64.82 ± 1.73
		Mean ADD		25.00	62.80	128.95	138.52	203.34
	Porcine		10.24 ± 0.19	14.18 ± 0.00	33.08 ± 2.36	70.88 ± 0.00	8.62 ± 0.47	62.63 ± 0.96
		Mean ADD		24.42	57.50	128.38	137.00	199.63
28.2	Equine		10.11 ± 0.25	14.80 ± 2.96	26.63 ± 0.00	62.14 ± 5.13	11.10 ± 1.82[a]	64.37 ± 3.39
		Mean ADD		24.91	51.54	113.68	124.78	189.15
	Porcine		9.62 ± 0.43	14.80 ± 2.96	32.56 ± 2.96	71.02 ± 5.13	10.26 ± 0.29	64.97 ± 1.15
		Mean ADD		24.42	56.98	128.00	138.26	203.23

Base temperature = 10°C. DD, degree day; ADD, accumulated degree days.
[a] $n = 2$.

stage of development than those in Byrd and Butler (1996). The only exceptions to this observation were for the egg stage at 28.2°C and the first instar stage at 20.8°C, both of which took less degree days to complete development in our study.

One factor that might explain the difference between the studies is that Byrd and Butler (1996) used oscillating temperature regimes for their 21.1°C and 26.7°C treatments, each with an amplitude of 5.5°C, to simulate naturally occurring conditions in Florida. Temperatures in our study were relatively stable. Behrens et al. (1983) determined that development of the cricket, *Gryllus bimaculatus*, De Geer (Orthoptera: Gryllidae), under oscillating temperature conditions was ≈26% faster than when reared under constant temperature conditions; however, it should be noted that in our study the mean temperatures and larval food taxon were different than those in Byrd and Butler (1996). In contrast, and more applicable to our study, Clarkson et al. (2004) determined that fluctuating versus constant temperatures delayed development of larvae of *Protophormia terraenovae* (Robineau-Desvoidy) (Diptera: Calliphoridae). Others, including Greenberg (1991), Byrd and Allen (2001), and as discussed throughout Byrd and Butler (1996), determined that development of blow flies reared under fluctuating temperatures was retarded in comparison with those reared under constant conditions, which is in contrast to our findings. However, it is important to note that these studies (Greenberg 1991, Byrd and Butler 1996, Byrd and Allen 2001) compared development for a single population of the target species within a given region. Our comparison with Byrd and Butler (1996) represents two unique geographic regions and potentially unique populations of *C. macellaria* (Gallagher et al. 2010).

Table 2. Sex ratio, degree days for longevity of male and female *Cochliomyia macellaria* ($n = 3$) on equine and porcine muscle tissue at three temperatures

Temperature (°C)	Tissue	Sex % ratio (♂:○)	Longevity (DD)	
			♂ ± SE	♀ ± SE
20.8	Equine	44.3:55.7	39.9 ± 1.7	39.9 ± 1.2
	Porcine	52.1:47.9	43.6 ± 0.2	43.3 ± 0.5
24.3	Equine	52.9:47.1	26.2 ± 4.7	31.2 ± 2.9
	Porcine	53.6:46.4	35.7 ± 2.2	36.2 ± 3.7
28.2	Equine	33.9:66.1	22.9 ± 0.5	22.2 ± 0.7
	Porcine	50.4:49.6	26.4 ± 1.3	26.8 ± 0.6

Base temperature = 10°C. DD, degree day.

Although largely untested, genetic variability may be an important source of development variability when comparing different populations (Tarone and Foran 2006). Several authors have generated development data for different populations of *L. sericata* (Kamal 1958, Greenberg 1991, Anderson 2000, Grassberger and Reiter 2001). Each of these populations is from a unique ecological setting, and the resulting development degree days differed. Kamal (1958), Greenberg (1991), and Grassberger and Reiter (2001) estimated faster minimum stage durations than Anderson (2000). Ames and Turner (2003) determined *Calliphora vicina* (Robineau-Desvoidy) (Diptera: Calliphoridae) and *Calliphora vomitoria* (Linnaeus) (Diptera: Calliphoridae) from different geographic locations have different thermal constant accumulated degree hour values.

A third factor explaining the variation observed between our study and Byrd and Butler (1996) is differences in laboratory protocols for rearing *C. macellaria.* Such factors include the difference in handling methods during rearing, as well as the use of different rearing chambers. Consequently, it is important to standardize rearing conditions, which best mimic growth on actual carrion to compare blow fly data sets and use them in legal investigations in different regions of the country (Tarone and Foran 2006). If such standardized protocols were developed by forensic entomologists, researchers could then focus their work toward understanding the development differences between populations that can be attributed to genetic variability.

Our study assessed the development of *C. macellaria* on equine and porcine striated muscle tissue. Although we observed no significant difference in

mean larval length or weight for specimens reared on equine and porcine muscle, Clark et al. (2006) determined in their study that the species origin on which *L. sericata* larvae were reared indeed influenced weight and length over time. However, Clark et al. (2006) determined that tissue source did have an effect on development time, which is similar to our results. They determined that *L. sericata* needed ≈31 h additional to complete the feeding stage and were 2 mm shorter when fed porcine liver rather than lung. Similarly, *C. vicina* fed porcine lung, heart, kidney, or brain completed the feeding stages 2 d sooner than those fed porcine liver (Kaneshrajah and Turner 2004). Consequently, until we have a better understanding of the variation in larval development as it relates to tissue fed upon, we recommend that such data sets be applied in a conservative manner when analyzing larval evidence collected from a living or deceased individual. Otherwise, generalizing larval development regardless of the tissue fed could result in an over- or underestimate of the larval age and, consequently, the period of insect activity.

Most research on the development of forensically important blow flies has focused on gathering data for the egg, larval, and pupal stages (Greenberg 1991, Grassberger and Reiter 2001, Tarone and Foran 2006). Although some data on adult longevity for such blow flies do exist (Gabre et al. 2005), our study is the first to break down adult longevity based on sex (Table 2). Our results indicate that males and females each live for comparable amounts of degree days after emergence. This information could be useful in abuse or death investigations in which empty pupal cases and dead adults are collected from indoor body recovery sites. This information could provide a conservative estimate of the time since emergence and, consequently, a more refined estimate of the period of insect activity using the degree day estimates.

Our study produced the second development data set for *C. macellaria* in the United States. These data offer new insight to variation in development across tissue types and temperatures as related to accumulated degree days, larval length, and weight. In addition, these data could be used to determine the period of insect activity for *C. macellaria* specimens collected from human, as well as livestock, remains in Texas. However, based on development differences observed between our study and Byrd and Butler (1996), additional studies are needed for other regions of the United States to gain a better understanding of this variation and its application in forensic investigations.

Acknowledgments

We thank K. Schoenly, J. Cammack, H. LeBlanc, A. Tarone, J. Byrd, R. Mohr, and three anonymous reviewers for their time and effort reviewing earlier versions of this manuscript.

References Cited

Amendt, J., C. P. Campobasso, E. Gaudry, C. Reiter, H. N. LeBlanc, and M.J.R. Hall. 2007. Best practice in forensic entomology: standards and guidelines. Int. J. Legal Med. 121: 90–104.

Ames, C., and B. Turner. 2003. Low temperature episodes in development of blowflies: implications for postmortem interval estimation. Med. Vet. Entomol. 17: 178–186.

Anderson, G. S. 2000. Minimum and maximum development rates of some forensically important Calliphoridae (Diptera). J. Forensic Sci. 45: 824–832.

Anderson, G. S., and N. R. Huitson. 2004. Myiasis in pet animals in British Columbia: the potential of forensic entomology for determining duration of possible neglect. Can. Vet. J. 45: 993–998.

Archer, M. 2007. Forensic entomology. Australian Police J. 61: 66–70.

Behrens, W., K. H. Hoffman, S. Kempa, S. Gäßler, and G. Merkel-Wallner. 1983. Effects of diurnal thermoperiods and quickly oscillating temperatures on the development and reproduction of crickets, *Gryllus bimaculatus.* Oecologia 59: 279–287.

Byrd, J. H., and J. C. Allen. 2001. The development of the black blow fly, *Phormia regina* (Meigen). Forensic Sci. Int. 120: 79–88.

Byrd, J. H., and J. F. Butler. 1996. Effects of temperature on *Cochliomyia macellaria* (Diptera: Calliphoridae) development. J. Med. Entomol. 33: 901–905.

Clark, K., L. Evans, and R. Wall. 2006. Growth rates of the blowfly, *Lucilia sericata* on different body tissues. Forensic Sci. Int. 156: 145–149.

Clarkson, C. A., N. R. Hobischak, and G. S. Anderson. 2004. A comparison of the development rate of *Protophormia terraenovae* (Robineau-Desvoidy) raised under constant and fluctuating temperature regimes. Can. Soc. Forensic Sci. 37: 95–101.

Gabre, R. M., F. K. Adham, and H. Chi. 2005. Life table of *Chrysomya megacephala* (Fabricius) (Diptera: Calliphoridae). Acta Oecologica 27: 179–183.

Gallagher, M. B., S. Sandhu, and R. Kimsey. 2010. Variation in development time for geographically distinct populations of the common green bottle fly, *Lucilia sericata* (Meigen). J. Forensic Sci. 55: 438–442.

Grassberger, M., and C. Reiter. 2001. Effect of temperature on *Lucilia sericata* (Diptera: Calliphoridae) development with special reference to the isomegalen- and isomorphen-diagram. Forensic Sci. Int. 120: 32–26.

Greenberg, B. 1991. Flies as forensic indicators. J. Med. Entomol. 28: 565–577.

Hall, D. G. 1948. The Blowflies of North America. Thomas Say Foundation, Baltimore, MD.

Harrison, B. A., and W. G. Pearson. 1968. A case of aural myiasis caused by *Cochliomyia macellaria* (Fabricius). Military Med. 133: 484–488.

Kamal, A. S. 1958. Comparative study of thirteen species of sarcosaprophagous Calliphoridae and Sarcophagidae (Diptera). I. Bionomics. Ann. Entomol. Soc. Am. 51: 261–271.

Kaneshrajah, G., and B. Turner. 2004. *Calliphora vicina* larvae grow at different rates on different body tissues. Int. J. Legal Med. 118: 242–244.

Nabity, P. D., L. G. Higley, and T. M. Heng-Moss. 2006. Effects of temperature on development of *Phormia regina* (Diptera: Calliphoridae) and use of development data in determining time intervals in forensic entomology. J. Med. Entomol. 43: 1276–1286.

SPSS. 2005. SPSS for Windows, release 14.0.1 computer program, version 14.0. SPSS, Chicago, IL.

Tarone, A. M., and D. R. Foran. 2006. Components of developmental plasticity in a Michigan population of *Lucilia sericata* (Diptera: Calliphoridae). J. Med. Entomol. 43: 1023–1033.

Williams, K. A., and M. H. Villet. 2006. A history of southern African research relevant to forensic entomology. S. African J. Sci. 102: 59–65.

Received 7 August 2009; accepted 16 June 2010.