Forensically Important Calliphoridae (Diptera) Associated with Pig Carrion in Rural North-Central Florida

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ABSTRACT A study to determine the relative abundance and seasonality of forensically important blow flies (Diptera: Calliphoridae) in rural north-central Florida was conducted using pig carcasses (*Sus scrofa* L.) as models for human bodies. Seven species of Calliphoridae were collected: *Lucilia coeruleiviridis* (=*Phaenicia*) (Macquart), *Cochliomyia macellaria* (F.), *Chrysomya rufifaces* (Macquart), *Phormia regina* (Meigen), *Chrysomya megacephala* (F.), and a few specimens of *Calliphora livida* Hall, and *Calliphora vicina* Robineau-Desvoidy. Species composition in aerial collections of adult flies, preserved larval collections, and samples of larvae reared to the adult stage were all highly correlated. Relative abundance of the species found was significantly different, with *L. coeruleiviridis* the most abundant species year-round. The relative abundance of the collected species varied significantly by day of decomposition and by season, with significant interactions between season and day, season and species, and day and species. *L. coeruleiviridis*, *C. macellaria*, *C. rufifaces*, and *P. regina* were found during the entire year, two *C. vicina* specimens and 11 *C. livida* specimens were collected from December to March, whereas *C. megacephala* was collected only from June through September.

KEY WORDS forensic entomology, successional pattern, postmortem interval

Forensic entomology is an extensive discipline where arthropod science and the judicial system interact. Forensic entomology is divided into three areas: medicocriminal entomology (also referred to as medicolegal entomology), urban entomology, and stored-product entomology (Hall 2001). Information gained from medicolegal entomology typically is used to determine the postmortem interval (PMI) (Gordh and Headrick 2001).

Succession is the orderly, predictable pattern of species replacement in an ecosystem (Ricklefs 1973, Price 1997, Gordh and Headrick 2001). Insects, especially the Calliphoridae or blow flies, and other invertebrates found on carrion, form a distinct faunal succession associated with the various stages of decay (Ash and Greenberg 1975; Lane 1975; Greenberg 1991; Catts and Goff 1992; Schoenly and Haskell 2000; Anderson 2001; Byrd and Castner 2001; Tenorio et al. 2003; Watson and Carlton 2003; Olivera-Costa and de Mello-Patiu 2004; Tabor et al. 2004, 2005; Watson and Carlton 2005; Amendt et al. 2006). Recognition of the different immature stages of each species involved, together with the knowledge of their rates of development, can give an indication of the PMI (Ash and Greenberg 1975; Smith 1986; Greenberg 1991; Greenberg and Kunich 2002; Amendt et al. 2004, 2007). Evaluation and interpretation of entomological evidence at a crime scene can identify other factors, including the season or geographic location at time of death, whether movement or storage of the remains occurred after death, the location of specific sites of trauma on the body, sexual molestation, or the use of drugs (Haskell et al. 1997).

In case studies conducted in varying temperate and tropical climates where human remains were exposed to the environment for 2.5 mo or less, entomology-based PMI estimates differed by ±48 h compared with the intervals determined by independent corroboration such as confessions and eyewitness testimony (Ash and Greenberg 1975, Greenberg 1985, Goff et al. 1988, Lord 1990, Byrd 1998). Entomological evidence is statistically the most reliable scientific means of estimating PMI compared with other methods such as police reports and autopsy results (Kashyap and Pillay 1989, Catts and Haskell 1990, Anderson 2001). The same entomological criteria used in determination of PMI for humans are applicable for deceased wildlife (Watson and Carlton 2003).

Few quantitative, statistically analyzed successional studies of forensically important calliphorids exist. Lack of funds and lack of awareness of the capabilities of forensic entomology for determination of PMI are just two of the reasons that successional studies are not undertaken. Most desirable would be a national database of the succession of forensically important entomological species in each ecoregion, especially for the calliphorids.

The objectives of this study were to determine the species succession of Calliphoridae associated with pig

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Table 1. Trial dates with mean high and low temperatures (°C ± SD), sample days where larval and adult collections were made, number of pigs, and mean weight kilograms of the pigs (±2.3 kg) during the study in Earleton, FL

Sample date	Mean high ± SD	Mean low ± SD	Sample days	No. pigs	Mean wt.
5–9 Feb. 2002	19.9 ± 4.1	8.1 ± 4.5	5	4	25
15–19 Mar. 2002	30.4 ± 1.2	16.5 ± 3.0	5	4	25
20–23 May 2002	25.9 ± .9	17.4 ± 3.2	4	4	30
22–25 July 2002	33.2 ± 1.3	22.4 ± 0.7	5	4	18
19–23 Aug. 2002	30.5 ± 2.9	23.9 ± 1.3	5	4	28
23–27 Sept. 2002	29.3 ± 3.2	23.7 ± 1.7	5	4	18
26–28 Oct. 2002	28.4 ± 2.2	19.1 ± 0.5	3	4	20
Nov.–14 Dec. 2002	16.8 ± 4.0	9.7 ± 4.3	15	4	22
30 Dec.–11 Jan. 2003	17 ± 3.3	5.7 ± 5.2	13	3	26
1–8 Mar. 2003	22.1 ± 6.2	16.9 ± 3.5	8	4	20
1–6 April 2003	25 ± 5.6	10.8 ± 5.8	6	4	18
26 April–1 May 2003	27.4 ± 3.9	18.4 ± 1.1	6	3	23
12–15 June 2003	30.3 ± 1.3	23 ± 1.1	4	3	22
8–20 Dec. 2003	17.4 ± 3.4	9.4 ± 4.0	13	3	27
23–31 Jan. 2004	18.9 ± 4.5	8.9 ± 6.6	9	3	16
5–14 Mar. 2004	24.5 ± 4.6	13.3 ± 4.5	10	3	27

carrion in rural north central Florida and how season affects the assemblage of calliphorid species.

Materials and Methods

Pig carcasses (*Sus scrofa* L.) were purchased from North Florida Livestock Market (Lake City, FL). Before purchase, the pigs were killed by the market owner by a shot into the top of the head with a 0.22 caliber rifle, resulting in the instant death of the animal. Each carcass was immediately double-bagged in a heavy-duty plastic trash bag and transported from Lake City to the study site, ≈96 km.

Study Site. The carcasses were located in a 48.6-ha section of north Florida flatwoods, 19.3 km east of Gainesville, near Earleton (29° 42′ N, 82° 06′ W) on the southeastern corner of N.E. SR 26 and SR1469. They were placed in wooded habitat where sunlight was somewhat restricted. In some cases, the pigs received direct sunlight during certain parts of the day, whereas at other times they were shaded. The study site mainly consisted of live oak, *Quercus virginiana* Mill.; slash pine, *Pinus elliottii* Engelman; and an understory of saw palmettos, *Serenoa repens* (Bartram) and grasses (Soil and Water Conservation Society 1989).

Data Collection. Trials were conducted from 5 February 2002 to 14 March 2004. Each trial consisted of three to four pigs placed at least 18 m apart, with trials conducted throughout the year at predetermined dates (Table 1). To prevent disturbance from scavengers, cages (90 cm in length by 60 cm in width by 50 cm in height) were constructed of wire mesh (5 by 5 cm) and placed over each pig. Cages were then secured with at least four bungee cords stretched tightly between the cages and tent stakes that were driven into the ground around the cage. Observations and collections were made daily if possible, during the afternoon when flies were most active, until maggot dispersal and pupation occurred. The pigs were not moved during the study, but the cages were lifted off for each sampling period.

Sampling Protocol. On day 1, the pigs were removed from the bags and placed on the ground at the research site, and the location was recorded with a hand-held Magellan GPS 315 global positioning system. After all the pigs were in position, data were collected including pig number, time of death, date, time of sample, sample number(s), ambient temperature, wind velocity, and a brief description of the weather (e.g., sunny, cloudy, rainy). A data logger (see description in Meteorological Measurements) was affixed to a nearby tree or bush, and the temperature probe was placed on the ground, in the shade, 1 to 2 meters from each pig. Therefore, it was the ground surface temperature—similar to that which the pig was exposed—that was being recorded.

On each day, aerial collections of adult flies were made over each pig with an insect net, with a minimum of 10 adult calliphorid flies sampled when possible. Flies were placed in vials with 70% isopropyl alcohol and later pinned and identified. From day 2 onward, one larval sample was taken from each maggot mass and split in half, with each half consisting of ≈25–250 larvae. The location where the sample was taken on the body was noted. Specimens for preservation were boiled in water for 2 min by using a camp stove and then placed in vials with 70% isopropyl alcohol. Live larvae were placed in small plastic containers with calves' liver for larval food and organic pupariation substrate. They were then reared to the adult stage to ascertain the identity and relative abundance of adult and third larval instars. Adult and larval specimens were identified based on morphological characteristics in taxonomic keys (Hall 1948, Hall and Townsend 1977).

Meteorological Measurements. During each sampling period, daily high temperatures were determined with a Taylor 9841 digital thermometer (Forestry Suppliers, Jackson, MS) shielded from direct rays of the sun. Ground surface temperatures were taken with HOBO (Onset Computer Corp., Bourne, MA) data loggers, set to record temperature every 30 min for 3 wk. Data logger temperatures were used for the daily low. Astronomical seasons are designated as follows; summer (22 June–21 September), fall (22 Sep-

tember–21 December), winter (22 December–21 March), and spring (22 March–21 June).

Data Analysis. The correlation among sampling methods (aerial, reared, and preserved collections) was tested with Pearson's coefficient and a two-tailed test (GraphPad Instant Biostatistics, GraphPad Software Inc., San Diego, CA). Sampling trials were compared by proportion of species collected daily during the sampling period. The number of flies caught in the aerial surveys, season, replicate number, day, and species were analyzed with fixed factor, repeated measures GLM analysis of variance (ANOVA) (NCSS, Kaysville, UT) (Price 1997). The independent monthly trials were grouped by season (spring, four trials; summer, two trials; fall, four trials; and winter, six trials), and counts were transformed [Log $(x + 0.015)$] to normalize variance (Price 1997).

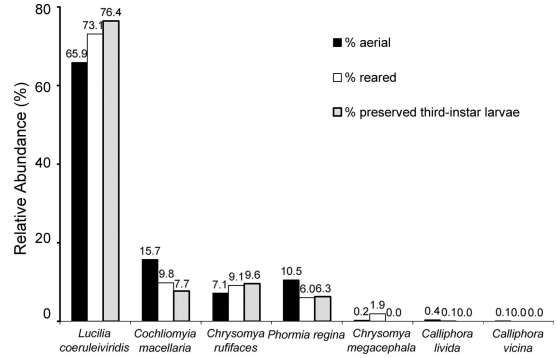

Fig. 1. Relative abundance of each species within the aerial collections versus reared specimens versus preserved third instars for the study in Earleton, FL.

Results

Diversity, Abundance, and Behavior. Seven calliphorid species were collected during the 16 trials. These species were, in descending order of abundance: *Lucilia coeruleiviridis* (Macquart), *Cochliomyia macellaria* (F.), *Chrysomya rufifaces* (Macquart), *Phormia regina* (Meigen), *Chrysomya megacephala* (F.), *Calliphora livida* Hall, and *Calliphora vicina* Robineau-Desvoidy (=*C. erythrocephala* Meigen). Calliphorid seasonality was characterized by a predominance of *L. coeruleiviridis* year-round. Of the total adult specimens (n = 6,263) identified during the study, 66.8% were *L. coeruleiviridis.* Similarly, of the identifiable third instars (n = 7,807), 76.4% were *L. coeruleiviridis. C. macellaria*, *C. rufifaces*, and *P. regina* also were found year-round, but in lower numbers. *C. megacephala* was found only during the hottest months of the year, from July to the end of October, and *C. livida* and *C. vicina* were found only during the coldest days of the year.

Except for the coldest days of the year, calliphorids occurred within the first 30 s after removing the carcasses from the plastic bags. During the first day of every trial, eggs were first oviposited in the natural orifices of the head and wound area. Once the eggs hatched, the larvae were concentrated in the head region, usually until no tissues remained, leaving just bones. The larval mass then progressed back to the abdominal region, sometimes leaving dried, skeletal remains of the head, whereas the rest of the carcass was actively decaying as larvae consumed the remaining tissues.

Relative abundance of each species within the aerial collections versus reared specimens versus preserved third instars for the study is depicted in Fig. 1. The correlation statistics between aerial and reared specimens were r = 0.9738, 95% CI = 0.8263–0.9962, P = 0.0003; between aerial and preserved specimens were r = 0.9826, 95% CI = 0.8820–0.9975, P = 0.0002; and between reared and preserved specimens were r = 0.9872, 95% CI = 0.9119–0.9982, P = 0.0001. Because their correlation was so strong, the larval samples (reared and preserved) were combined to compute species proportion of larvae for daily and seasonal succession. Data analysis for adults (Table 2) indicated that species, season, and day were highly significant and that interactions between season and day, season and species, and day and species were highly significant.

Colonization of Carcasses in Spring (Fig. 2). In all replicates, *L. coeruleiviridis* adults were the first to arrive at the carcass. All four species of adult flies collected in the spring were present from day 1 through day 4 at the carcass, with *L. coeruleiviridis* the most abundant species. By day 4, maggot masses generally consisted of all four species of calliphorids: *L. coeruleiviridis*, *C. macellaria*, *C. rufifaces*, and *P. regina.*

Table 2. GLM ANOVA table for adult specimens collected during the study in Earleton, FL

Source term	df	Sum of squares	Mean square	F ratio	Prob. level	Power (α = 0.05)
A: season	3	39.727	13.242	13.59	0.0004*	0.9976
B(A): replicate	12	11.689	0.974	1.02	0.4261	
C: day	4	30.330	7.583	7.97	<0.0001*	0.9981
AC	12	30.399	2.533	2.66	<0.0001*	0.9810
D: species	4	112.863	28.216	29.66	<0.0001*	1.0000
AD	12	50.158	4.180	4.39	<0.0001*	0.9998
CD	16	45.573	2.848	2.99	<0.0001*	0.9983
S	336	319.642	0.951			
Total (adjusted)	399	652.247				
Total	400					

*, significant at 0.05.

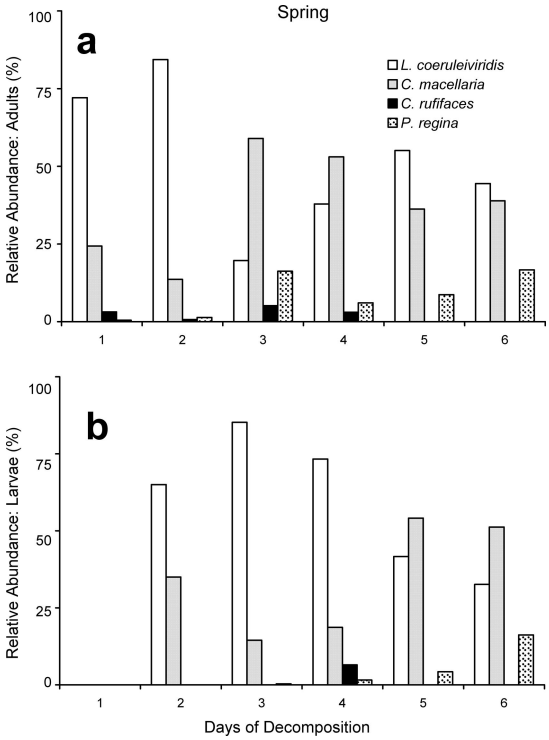

Fig. 2. Spring daily relative proportions of calliphorid adults (a) and larvae (b) associated with pig carrion in Earleton, FL.

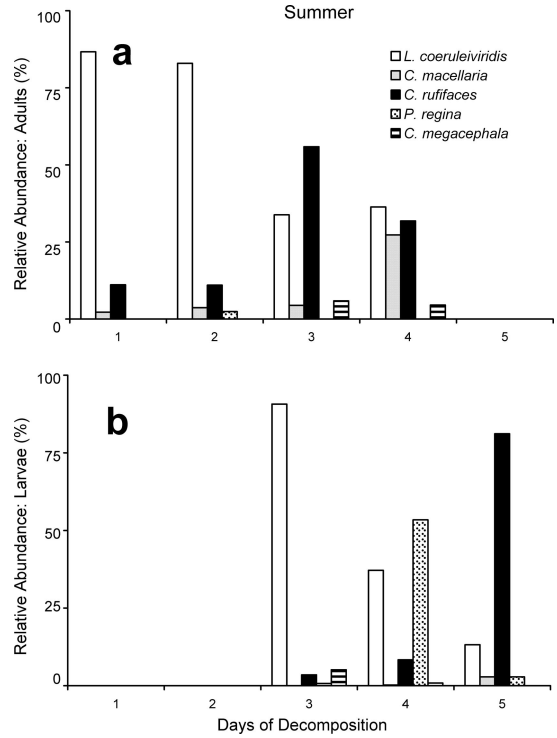

Fig. 3. Summer daily relative proportions of calliphorid adults (a) and larvae (b) associated with pig carrion in Earleton, FL.

Colonization of Carcasses in Summer (Fig. 3). It rained heavily during all of the field trials in the summer, possibly affecting the adult catches. Five species of calliphorid flies were collected: *L. coeruleiviridis*, *C. macellaria*, *C. rufifaces*, *P. regina*, and *C. megacephala.* Again, *L. coeruleiviridis* was the first to arrive at the carcasses, making up almost 90% of all adults captured on days 1 and 2, but a small proportion of *C. macellaria* and *C. rufifaces* also were collected on those days (Fig. 3a). After day 3, *L. coeruleiviridis* proportion of larvae declined rapidly, so that on day 4, *P. regina* was the most abundant species (Fig. 3b). *C. megacephala* larvae were collected on days 3 and 4 in small numbers. We did not collect *C. macellaria* larvae until day 5 during the summer, when most of the other larvae had already migrated off the pig. This differs from the spring data, which showed that *C. macellaria* were present every day during the first 6 d.

Colonization of Carcasses in Fall (Fig. 4). Five species of calliphorid flies were collected during the fall: *L. coeruleiviridis*, *C. macellaria*, *C. rufifaces*, *P. regina*, and *C. megacephala. L. coeruleiviridis* was again the most abundant species of fly during the fall followed by *C. macellaria* (Fig. 4a). *L. coeruleiviridis* were overwhelmingly the most abundant species collected during the entire sampling period (Fig. 4b). *C. megacephala* larvae were collected on days 4 and 5 only, although no adults of this species were captured in the fall trials.

Colonization of Carcasses in Winter (Fig. 5). Six species of calliphorids were collected during the winter: *L. coeruleiviridis*, *C. macellaria*, *C. rufifaces*, and *P. regina*, plus two *C. vicina* specimens and 11 *C. livida* specimens. The last two species are not included in the figure because they represented such a small proportion of flies collected. *L. coeruleiviridis* was again the most abundant species of calliphorid. The second most abundant calliphorid during the winter was *P. regina.* The proportions of *L. coeruleiviridis* adults decreased and *P. regina* adults increased steadily until by day 7 (Fig. 5a). *L. coeruleiviridis* larvae were overwhelmingly the most abundant species of larvae every day during the winter (Fig. 5a and b).

Discussion

L. coeruleiviridis is a necrophagous species and an important forensic indicator. It was the most abundant calliphorid species collected on pig carrion during this study. This species was always the first to arrive at fresh pig carrion, the first to deposit eggs, the first to complete development, and the first to migrate off the carcass to pupariate in the soil. In spring and summer, these events took 6 d or less to complete. This species was the most abundant in the cooler months from late October to the end of May. It was present, but in smaller numbers, during the summer when the temperatures were >25.0°C. *P. regina*, *C. macellaria*, and *C. rufifaces* were collected during all seasons as well,

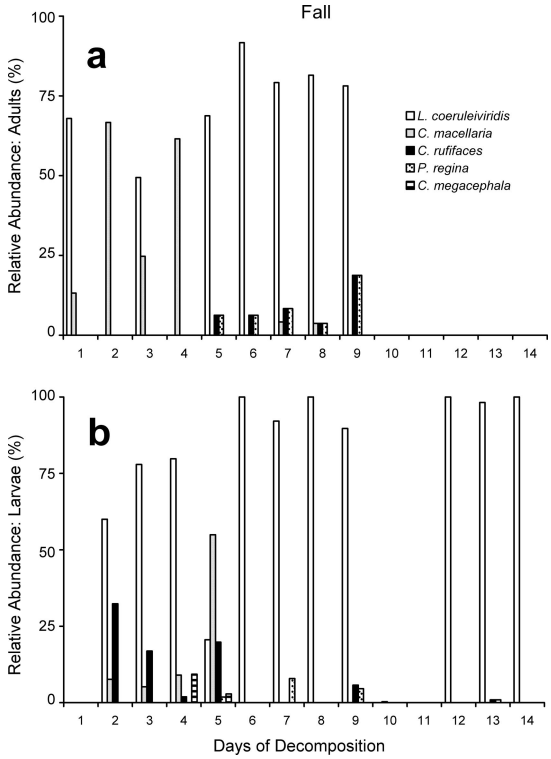

Fig. 4. Fall daily relative proportions of calliphorid adults (a) and larvae (b) associated with pig carrion in Earleton, FL.

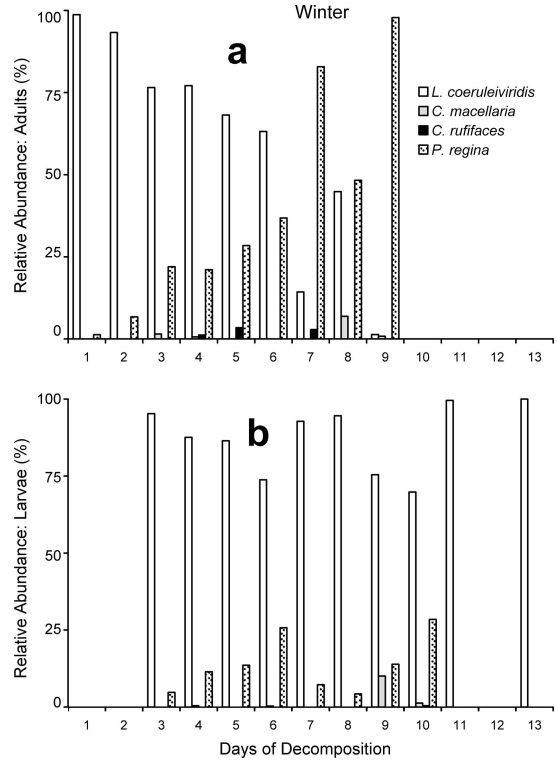

Fig. 5. Winter daily relative proportions of calliphorid adults (a) and larvae (b) associated with pig carrion in Earleton, FL.

and again mostly in the cooler months. Hall (1948) reported that *P. regina* is abundant in the spring in the southern states (location not defined), and Byrd (2001) reported that *P. regina* is not usually found during the summer in Florida. However, we did find this species during the summer in our study, but in small numbers compared with the other seasons.

C. megacephala were collected only during the hottest months of the year when the carcasses had been decomposing for at least 3 d. Pérez et al. (2005) noted that *C. megacephala* in Columbia, South America, did not arrive at pig carcasses until the third day of decomposition. Death scene photographs from Alachua and Duval counties, FL, indicated that *C. megacephala* were present on humans within hours of death (S.V.G., personal observation). J. Byrd (personal communication) also viewed *C. megacephala* on human remains less that 24 h postmortem in several counties in Florida. Byrd and Butler (1997) noted that *C. megacephala*, an introduced species, has the potential to displace native calliphorid species and that it could become an important forensic indicator in this region. The apparent differences in occurrence of *C. megacephala* on pig carcasses compared with human carcasses indicates the need for replicated studies comparing arrival times of calliphorid flies on human cadavers and pigs, especially in places such as Florida, where exotic insect species continue to arrive.

Although the proportions of adults collected changed during the decomposition process, *L. coeruleiviridis*, *C. macellaria*, *C. rufifaces*, and *P. regina* were all present on the first day during the spring and summer. In the spring, presence of *L. coeruleiviridis* and *C. macellaria* larvae on day 2 indicated that these species were ovipositing immediately or soon after arriving, because the pigs were laid out in the afternoon with only a few hours of daylight remaining. The presence of *P. regina* on day 3 onward indicated a possible delay in egg laying for this species (Hall and Doisy 1993). The stronger presence of adult *P. regina* on day 3 to day 6 coincides with the increase in larvae collected on the same days.

As noted above, during the summer trials it rained heavily almost every day. The ground was very wet, and parts of the study site were under water, although the pigs were never under water at any time. It is possible that eggs were washed off during these heavy downpours or that the rains prevented females from depositing eggs. Despite the rain, the temperatures were very hot (between 32.2 and 37.8°C), and adult flies were sometimes caught during light rain. Based upon presence of adults, rain and high temperatures, it is likely that *L. coeruleiviridis*, *C. rufifaces*, *P. regina*, and *C. megacephala* larvae collected on day 3 resulted from adult oviposition on day 2. We do not know whether this apparent delay in larval activity was caused by high ambient temperatures, torrential rainfall, decomposition stage of the pig (therefore, attrac-

tancy), presence of predacious *C. rufifaces* larvae, or possibly other factors.

During the fall, it seemed that *L. coeruleiviridis*, *C. macellaria*, and *C. rufifaces* larvae collected on day 2 resulted from oviposition on day 1, a strong indication that these species located and oviposited on the carcass soon after death. The temperatures in the fall can reach up to 28°C during the day, yet they can decrease to 19°C at night. This cooling off at night during the fall results in slower carcass decomposition rate. During the spring and summer, maggot masses were completely dispersed by day 7, but in the fall, large quantities of larvae were present for ≈2 wk, and *L. coeruleiviridis* larvae dominated the carcasses. As in the summer, *C. megacephala* larvae were only collected on days 4 and 5, when the carcasses were well into the decay process. Also, *P. regina* adults and larvae were not collected until day 5, indicating that they were either not present, or present only in small numbers and not sampled during the first days. This contrasts with the summer and winter collections, where this species was collected from the beginning with *L. coeruleiviridis*, but it is consistent with the spring collections, where the adults were present from the beginning, but the larvae were not collected until 1 d after *L. coeruleiviridis.*

During the winter, *L. coeruleiviridis* and *P. regina* larvae were collected starting on day 3. We did not expect to collect larvae on day 1 or 2 because of the cold ambient temperatures. The presence of *C. livida* on the first 4 d may indicate a preference for fresh carrion. *P. regina* adults and larvae were present until day 10 of the decomposition process, clearly indicating that they are able to consume fresh as well as aging (highly decomposed) carrion.

During a trial that took place in April–May 2003, a large maggot mass moved away from the pig, and over the course of 7 d, the large mass split into two separate postfeeding maggot masses. One large mass contained three species: *L. coeruleiviridis*, *C. macellaria*, and *P. regina*, and the other, smaller mass contained only *C. rufifaces.* A few *L. coeruleiviridis* maggots dropped into the *C. rufifaces* mass were instantly consumed by the *C. rufifaces* larvae. This separation of mass by species was seen only once during the duration of the study.

Byrd (1998) collected *Lucilia cuprina* (Wiedemann) (=*Phaenicia pallescens* Shannon) from pig carcasses in Gainesville, FL, from June to September 1996, and also in September 1997, but this species was not found during this study. *L. cuprina* is considered an urban fly that prefers excrement to carrion (Byrd 2001), which could explain why it was not found during this study in rural Florida.

We also did not collect *Lucilia sericata* (Meigen) and *Lucilia eximia* (Wiedemann) in rural north-central Florida, whereas Byrd (1998) did collect specimens in Gainesville, FL. Different calliphorid species have been associated with different habitats, and in urban areas (with odors from human refuse, cooking, garbage dumps, and improper sanitation) can attract different species assemblages than rural, wooded areas, or arid regions (Anderson 2001). Smith (1986) indicated that *C. vicina* is the most common calliphorid found on human corpses in urban areas; however, it was not found during this study.

One of the findings in this study was the high degree of correlation between aerial samples and larval collections. An aerial sample taken over a corpse when the flies are most active may therefore be a reliable indicator of what species are currently colonizing the corpse, although additional ground and larval sampling may be necessary at a death scene if much time has passed since death to properly sample the first arrivers. Except for the delayed arrival of *C. megacephala* in the summer and fall, *L. coeruleiviridis*, *C. macellaria*, *C. rufifaces*, and *P. regina* were present from the beginning to the first wave of larval migration during the decomposition process; thus, it was the relative proportion of species that changes. This indicated the need for large sample size for accurate determination of PMI.

There are no published rearing data for *L. coeruleiviridis* and forensic entomologists historically have used *L. sericata* rearing data, assuming that the data will be similar as they are closely related species. Taking into consideration that *L. coeruleiviridis* was the most common species in our study, and it is one of the most common on animal corpses in approximately one third of the United States (according to published literature), it is surprising that development data are not yet available for this species.

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