

## Minimum and Maximum Development Rates of Some Forensically Important Calliphoridae (Diptera)

**REFERENCE:** Anderson GS. Minimum and maximum development rates of some forensically important Calliphoridae (Diptera). *J Forensic Sci* 2000;45(4):824–832.

**ABSTRACT:** Blow fly development rates are frequently used to estimate elapsed time since death in homicide investigations in the first few weeks after death. However, in order to make more precise estimates of time since death, accurate developmental data must be generated for all carrion species, and at temperatures that are comparable with those found at crime scenes. This paper presents developmental rates determined for five forensically important species in British Columbia, *Phormia regina* (Meigen), *Phaenicia sericata* (Meigen), *Eucalliphora latifrons* (Hough), *Lucilia illustris* (Meigen), and *Calliphora vicina* Robineau-Desvoidy, at several temperatures.

**KEYWORDS:** forensic science, Calliphoridae, *Phormia regina*, *Phaenicia sericata*, *Eucalliphora latifrons*, *Lucilia illustris* and *Calliphora vicina*, forensic entomology, developmental rates

Blow flies (Diptera: Calliphoridae) are ubiquitous insects that impact on man and animals in many ways. Many species are nuisance pests and mechanically transmit a variety of human and animal diseases (1,2) as well as cause tremendous economic loss in sheep-rearing areas of the world due to myiasis or fly strike (3–5). Some species can also cause myiasis in humans, under appropriate conditions (6–10). For these reasons, and perhaps due to their attraction to carrion, garbage, and feces, together with the appearance of the immature stages, they are frequently considered highly undesirable insects.

However, blow flies can also be considered beneficial insects in many ways. Their immature stages, although perhaps unattractive, are vital decomposers in the degradative succession of carrion in the wild, rapidly consuming carcasses, thereby removing possible disease sources and recycling nutrients. In the early part of this century, the immature stages of several species of Calliphoridae were used in the extremely successful treatment of osteomyelitis in children, a disease that was previously untreatable before the advent of antibiotics (11). Although this treatment lost favor for a while after antibiotics were discovered, maggot therapy is now being reintroduced in many areas of North America and is used in cases of severe gangrenous infections and when surgical intervention, or other more conventional treatments are not possible (12–15). In some

cases, blow flies have even been used as pollinators of agricultural crops in controlled areas (16–18). However, perhaps the most unique beneficial application of the blow fly is the use of its life cycles and development in criminal investigations to determine time of death in homicide cases (19–26).

Previous studies on some species of Calliphoridae have shown conflicting developmental times and have been approached from different perspectives (22,26–28) and were also often performed at temperatures much higher than those found in forensic cases in British Columbia (personal observations). In some cases, there are few developmental data available for certain species (24). Therefore, this work was done to determine rates of development for some of the most common species found on carrion (29–35) and in human death investigations (19) in British Columbia.

### Methods and Materials

Wild adult Calliphoridae were collected in the Lower Mainland region of British Columbia. Inverted cone traps were used that consisted of a hollow metal canister (~20 cm high × 15 cm in diameter), open at both ends, containing a metal wire mesh cone, open at its apex, and attached to the inside of the canister along its rim. A clear plastic bag was secured over the top of the canister with an elastic band. The trap was supported on cork “legs” approximately 5 to 8 cm above a beef liver bait. The traps were placed outside in the early morning, in a variety of habitats and collected in the early evening. Adult flies were attracted to the protein source and, after feeding, flew directly upwards, into the canister, through the small opening at the apex of the mesh cone, and into the plastic bag. They were unable to find the small opening in the mesh cone to escape. The five species vary in their preferred habitats (19,36) and so trapping was performed in a number of areas.

*Calliphora vicina* Robineau-Desvoidy was collected primarily on the Simon Fraser University campus, which is situated on Burnaby Mountain in Burnaby, close to Vancouver, British Columbia. Endowment lands consisting of coniferous and deciduous forest surround the campus area, which is then surrounded by the city of Burnaby. Some specimens were collected in Aldergrove, a rural area of the Fraser Valley. This site, although close to a residence, is in an agricultural area, with mixed farming, and is close to forested areas.

*Phormia regina* (Meigen) was collected in a rural, forested area in Maple Ridge. This area is a research forest and is at least 25 km from any residential areas. Most specimens were collected as larvae or eggs from animal carcasses.

*Phaenicia sericata* (Meigen) was collected primarily near the university campus, and in the city of Burnaby itself, in a residential

<sup>1</sup> Assistant professor, School of Criminology, Simon Fraser University, Burnaby, B.C., Canada, and forensic entomology consultant to Royal Canadian Mounted Police and Municipal Police.

Received 5 Apr. 1999; and in revised form 26 July, 23 Sept. 1999; accepted 27 Sept. 1999.

area. Large numbers were collected in these areas, although a small number were collected in the more rural site in Aldergrove.

*Eucalliphora latifrons* (Hough) was collected on the university campus, at Simon Fraser University, Burnaby.

*Lucilia illustris* (Meigen) was collected primarily in the rural area, in Aldergrove. Large numbers were collected at this site, but some specimens were also collected on the university campus.

Wild adults were continuously added to the colonies to ensure that they did not become adapted to the lab conditions and new colonies were established each spring.

Adult flies were maintained in 75 cm<sup>3</sup> rearing cages. Each cage of insects was given water, sugar, and milk powder *ad libitum*. Three days after emergence, adult flies were given beef liver daily to allow for development of the ovaries. When eggs were required, fresh beef liver was placed in the cage for oviposition, and checked for the presence of eggs every 15 to 30 min. As soon as one female began to lay eggs, many females would mass on the liver and lay large egg batches. The time of oviposition was noted.

Each experiment consisted of two 1 gallon (4.5 L) wide-mouthed glass jars. The base of each was lined with 5 cm of slightly dampened sawdust, followed by a folded paper towel. Approximately 500 gm of fresh beef liver was placed on the paper towel, and approximately 200 eggs were removed from the oviposition media to the fresh liver. The jar was closed with two layers of paper towel, secured with several elastic bands. Each jar in an experiment was set up identically and was then arbitrarily assigned to a control or experimental group. The insects were then raised at several controlled temperatures in a Forma Scientific refrigerated incubator. Fresh beef liver was added as required, and was always in excess so that food was not a limiting factor. Temperature was monitored using SmartReader 1<sup>®</sup> dataloggers, programmed to record temperature every 30 min. *C. vicina*, *P. sericata*, and *E. latifrons* were raised at three controlled temperatures, ~16°C, 21°C, and 23°C (exact temperature varied with species). Several experiments were conducted under each temperature regime. *P. regina* was raised at 16°C and 23°C. *L. illustris* was only raised at one temperature, 21.1°C, as this species proved difficult to maintain in the lab beyond 1 to 2 generations. Three species, *C. vicina*, *P. sericata*, and *E. latifrons* also were raised under natural, fluctuating, temperature regimes, outside. They were placed in a shaded area and protected from rain but were exposed to normal temperature variations.

**Experimental Jar**—The egg mass was examined every few hours until eclosion was complete. Eggs were oviposited late in afternoon/early evening so that hatch was unlikely to occur over the night time. They were then checked to determine whether they were still eggs the next morning (i.e., none had hatched overnight—none ever did, due to timing of oviposition) then observed every 1 to 2 h until hatch began, then until hatch was complete. Times of first and last eclosion were noted. Following eclosion, the larvae were examined at regular intervals to determine size and developmental stage. In the early stages, the insects were examined 3 to 4 times a day to determine length of time of each stage as accurately as possible. When larvae were noted to be about to molt into the next stage they were checked very frequently until all had entered the next stage. When the insects were in the third instar and beyond, they were examined only 2 to 3 times per day because developmental times are longer. At each examination time, a representative sample of 20 specimens was removed from the jar and physically examined. Each larva was measured and examined under a binocular microscope to determine developmental stage. Examination of each insect was brief, and the insects were not

chilled or in any way treated at this time, and all were returned to the jar. The insects were measured as they crawled, at full extension. First, second, and third instars were distinguished principally on the number of posterior spiracular slits (37) and from readiness to molt. The third instar is divided into two, behaviorally separate, stages. The first stage is the feeding stage, during which the maggot feeds voraciously and the crop reaches the maximum size. At a given point, the larva ceases feeding and begins to utilize the contents of the crop, which gradually reduces until it is no longer visible. During this post-feeding or prepupal stage of the third instar, the insect generally leaves the food source and wanders into the soil and, in the case of a human body, the carpet or clothing (38,39,19). The color of the larva changes from translucent to opaque, by the presence of fat bodies; internal features become obscured by the fat bodies and eventually the larva shortens slightly preparatory to pupation (40). These features were all used to determine stage.

Preliminary experiments had suggested that, after the insects had entered this prepupal or post-feeding stage, they appeared to be somewhat disturbed by physical examination, unlike other stages, which did not appear to be affected. Therefore, once it was ensured that most larvae had entered the prepupal stage, they were no longer handled, but only visually examined to minimize the potential effects of handling.

Larvae were allowed to pupate in the sawdust and the jar was examined for adult emergence. Adults emerged into the jar, and were removed each day until all had emerged or died.

**Control Jar**—The control was used to determine the effect, if any, of handling a proportion of the insects on development and mortality. The egg mass was examined every few hours until eclosion, as in the experimental jar, as this could be done with no handling and little or no disturbance. However, after eclosion, the insects were only examined visually. This allowed visual comparisons of size, stage, and behavior, but did not disturb the insects. However, when the specimens were under the liver, or in a mass, no comparison could be made. The control insects were visually assessed at the same examination times as the experimental group. As this group was only visually assessed, time of molting from first to second instar, and from second to third, could not be determined. However, time of eclosion could be determined, as well as time at which the insects entered the prepupal stage of the third instar, time of pupation, and time of adult emergence.

Times of adult emergence in disturbed and control jars were compared using a two-tailed paired t-test (Microsoft Excel<sup>®</sup>) to determine the effects, if any, of physical handling on development rates. For each experiment, actual minimum percent time spent in a given developmental stage was determined. Then the mean minimum percent time spent in each developmental stage for all experiments conducted with that species, under that temperature regime, was determined. Two-factor analysis of variance (ANOVA), without replication (Microsoft Excel<sup>®</sup>) was used to determine differences, if any, between mean percent time (minimum) spent in each stage between species and between temperatures ( $\alpha \leq 0.05$ ).

## Results

In all cases, irrespective of species or temperature, there were no significant differences between total minimum developmental rates of insects in the control jars, which were not handled, and those in the experimental jars, in which a random sample was physically examined at each examination time ( $p \geq 0.2$ ). Therefore, data were pooled for a given temperature and species.

Table 1 shows the minimum and maximum time, in hours, for *P. regina* to reach each developmental stage at  $16.1 \pm 0.01^\circ\text{C}$  and  $23.0 \pm 0.02^\circ\text{C}$ . Three replicates were run at each temperature, each consisting of two jars of insects. As can be seen from Table 1 and as expected, *P. regina* developed more rapidly at the higher temperature. This species took almost twice as long to complete each stage of development, and to complete the entire cycle at  $16.1^\circ\text{C}$  than at  $23.0^\circ\text{C}$ . Also, development was more synchronous at the higher temperature, with minimum and maximum time to reach a given stage being very close, whereas, at the lower temperature, molt in each cohort was more spread out.

*P. regina* adapted readily to lab conditions and easily produced large quantities of viable eggs, after a liver meal. On one occasion, a larval colony was accidentally allowed to run out of liver. Development continued at the same rate as experimental colonies and adult emergence occurred at the same time. The adults from this starved colony were much smaller than those raised with an excess of larval medium, although longevity and fecundity did not appear to be affected.

Table 2 shows the minimum and maximum time taken for *C. vicina* to reach each developmental stage at  $15.8 \pm 0.004^\circ\text{C}$ ,  $20.6 \pm 0.03^\circ\text{C}$ , and  $23.3 \pm 0.02^\circ\text{C}$ . Seven replicates were run at  $15.8 \pm$

$0.004^\circ\text{C}$  (included one extra control jar), six at  $20.6 \pm 0.03^\circ\text{C}$ , and four at  $23.3 \pm 0.02^\circ\text{C}$ . Developmental rate increased as temperature increased, with minimum time to reach each stage taking between 1.5 and 2 times longer at  $15.8^\circ\text{C}$  as at  $23.3^\circ\text{C}$ . Overall, development took 1.7 times as long at the lower temperature. Minimum and maximum times to enter each stage at each temperature appeared similar until the prepupal and adult stages. At these stages entrance into the prepupal stage and adult eclosion was very spread out. As with *P. regina*, *C. vicina* readily adapted to lab conditions.

Table 3 shows the minimum and maximum time taken for *E. latifrons* to reach each developmental stage at  $15.8 \pm 0.005^\circ\text{C}$ ,  $21.0 \pm 0.04^\circ\text{C}$ , and  $23.3 \pm 0.02^\circ\text{C}$ . Eight replicates were run at  $15.8 \pm 0.005^\circ\text{C}$ , nine at  $21.0 \pm 0.04^\circ\text{C}$ , and only two at  $23.3 \pm 0.02^\circ\text{C}$ . As there was only one measured set of data for time of entry into second and third instar, and into the prepupal stage, no standard error is given for these times at  $23.3^\circ\text{C}$ . Developmental rate increased as temperature increased, with minimum time to reach each stage taking between 1.4 and 2.3 times longer at  $15.8^\circ\text{C}$  as at  $23.3^\circ\text{C}$ . Overall, development took 1.7 times as long at the lower temperature. Temperature did not appear to affect the synchronicity of molt. *E. latifrons* also appeared to readily adapt to lab conditions, and was simple to raise.

TABLE 1—Development data for *Phormia regina* (mean  $\pm$  standard error,  $\alpha = 0.05$ ).

Stage	$16.1 \pm 0.01^\circ\text{C}$ Time to Reach Stage, h		$23.0 \pm 0.02^\circ\text{C}$ Time to Reach Stage, h	
	Min	Max	Min	Max
1st instar	$43.0 \pm 1.9$	$48.3 \pm 1.1$	$21.5 \pm 0.9$	$22.5 \pm 0.5$
2nd instar	$159.0 \pm 11.0$	$174.3 \pm 22.9$	$78.8 \pm 6.1$	$85.0 \pm 6.0$
3rd instar (feeding stage)	$260.7 \pm 25.3$	$297.3 \pm 28.4$	$126.7 \pm 9.7$	$143.0 \pm 3.0$
Prepupal	$367.6 \pm 34.8$	$416.8 \pm 33.7$	$191.7 \pm 0.3$	$213.3 \pm 1.1$
Pupal stage	$440.8 \pm 20.8$	$525.8 \pm 26.2$	$217.5 \pm 4.6$	$268.3 \pm 11.0$
Adult	$716.3 \pm 16.4$	$863.3 \pm 24.0$	$369.3 \pm 3.7$	$434.8 \pm 11.1$

TABLE 2—Development data for *Calliphora vicina* (mean  $\pm$  standard error,  $\alpha = 0.05$ ).

Stage	$15.8 \pm 0.004^\circ\text{C}$ Time to Reach Stage, h		$20.6 \pm 0.03^\circ\text{C}$ Time to Reach Stage, h		$23.3 \pm 0.02^\circ\text{C}$ Time to Reach Stage, h	
	Min	Max	Min	Max	Min	Max
1st instar	$41.4 \pm 1.2$	$46.7 \pm 0.6$	$22.5 \pm 0.2$	$36.0 \pm 4.0$	$21.0 \pm 0$	$29.5 \pm 5.2$
2nd instar	$83.0 \pm 10.0$	$88.3 \pm 12.7$	$57.0 \pm 6.0$	$57.0 \pm 6.0$	$45.0 \pm 0$	$52.0 \pm 0$
3rd instar (feeding stage)	$128.0 \pm 9.0$	$146.0 \pm 15.6$	$84.0 \pm 10.0$	$93.5 \pm 0.5$	$77.0 \pm 0$	$85.0 \pm 8.0$
Prepupal	$228.0 \pm 3.3$	$257.0 \pm 9.6$	$155.5 \pm 4.2$	$162.5 \pm 1.1$	$146.0 \pm 0$	$173.0 \pm 0$
Pupal stage	$294.0 \pm 4.7$	$440.3 \pm 42.1$	$213.0 \pm 4.5$	$233.0 \pm 0.9$	$202.8 \pm 5.8$	$279.0 \pm 22.5$
Adult	$719.7 \pm 6.0$	$874.6 \pm 20.7$	$514.8 \pm 3.7$	$572.0 \pm 10.0$	$454.0 \pm 6.0$	$499.5 \pm 7.5$

TABLE 3—Development data for *Eucalliphora latifrons* (mean  $\pm$  standard error,  $\alpha = 0.05$ ).

Stage	$15.8 \pm 0.005^\circ\text{C}$ Time to Reach Stage, h		$21.0 \pm 0.04^\circ\text{C}$ Time to Reach Stage, h		$23.3 \pm 0.02^\circ\text{C}$ Time to Reach Stage, h	
	Min	Max	Min	Max	Min	Max
1st instar	$43.4 \pm 1.1$	$47.1 \pm 0.3$	$21.8 \pm 0.6$	$28.2 \pm 2.7$	$18.5 \pm 0$	$20 \pm 0$
2nd instar	$71.0 \pm 2.1$	$71.0 \pm 2.1$	$49.7 \pm 7.7$	$49.7 \pm 7.7$	42.5	42.5
3rd instar (feeding stage)	$104.7 \pm 5.7$	$136.0 \pm 17.6$	$77.7 \pm 9.7$	$94.7 \pm 1.2$	74	74
Prepupal	$201.1 \pm 5.3$	$206.8 \pm 7.1$	$148.0 \pm 4.1$	$159.3 \pm 3.2$	$116 \pm 0$	$143 \pm 0$
Pupal stage	$249.6 \pm 5.3$	$295.9 \pm 4.2$	$196.7 \pm 3.7$	$287.3 \pm 13.6$	$157 \pm 14$	$183 \pm 12$
Adult	$608.0 \pm 10.1$	$683.1 \pm 11.2$	$442.8 \pm 5.5$	$542.9 \pm 9.9$	$349.5 \pm 10.5$	$397.5 \pm 12.5$

TABLE 4—Development data for *Phaenicia sericata* (mean  $\pm$  standard error,  $\alpha = 0.05$ ).

Stage	15.8 $\pm$ 0.005°C		20.7 $\pm$ 0.03°C		23.3 $\pm$ 0.02°C	
	Time to Reach Stage, h		Time to Reach Stage, h		Time to Reach Stage, h	
	Min	Max	Min	Max	Min	Max
1st instar	40.6 $\pm$ 2.8	44.4 $\pm$ 1.1	20.9 $\pm$ 0.4	23.6 $\pm$ 0.6	21	22
2nd instar	94.3 $\pm$ 1.9	103.3 $\pm$ 8.0	52.3 $\pm$ 1.7	52.3 $\pm$ 1.7	45	45
3rd instar (feeding stage)	135.7 $\pm$ 6.7	158.7 $\pm$ 19.7	78.3 $\pm$ 9.3	95.3 $\pm$ 0.9	77	77
Prepupal	233.7 $\pm$ 4.3	246.7 $\pm$ 6.9	127.9 $\pm$ 3.3	145.3 $\pm$ 4.3	145	159.5
Pupal stage	382.3 $\pm$ 10.2	...*	245.7 $\pm$ 6.1	356.9 $\pm$ 14.6	264	...†
Adult	775.0 $\pm$ 18.7	917.2 $\pm$ 21.9*	486.2 $\pm$ 4.7	647.8 $\pm$ 18.0	468.5	624.5

\* At 15.8°C many specimens entered diapause in the prepupal stage and did not progress beyond this stage.

† At 23.3°C a few specimens entered diapause in the prepupal stage and did not progress beyond this stage.

Table 4 shows the minimum and maximum time taken for *P. sericata* to reach each developmental stage at 15.8  $\pm$  0.005°C, 20.7  $\pm$  0.03°C, and 23.3  $\pm$  0.02°C. Eight replicates were run at 15.8  $\pm$  0.005°C, nine at 21.0  $\pm$  0.04°C, and only two at 23.3  $\pm$  0.02°C. As there was only one measured set of data for time of entry into second and third instar, and into the prepupal stage, no standard error is given for these times at 23.3°C. Developmental rate increased as temperature increased, with minimum time to reach each stage taking between 1.4 and 2.1 times longer at 15.8°C as at 23.3°C. Overall, development took 1.8 times as long at the lower temperature. Temperature did not appear to affect the synchronicity of molt.

*P. sericata* reproduced readily in lab conditions, although this species was notable in its obvious attempts to escape from the colony jars during the prepupal stage. All the other species examined appeared to pupate willingly within the confines of the one gallon jar, in 5 cm of sawdust, in close proximity to the larval food source. *P. sericata*, on the other hand, would crawl, *en masse* up the glass sides of the jar, and attempt to push the paper toweling off the jar. In some cases this resulted in the elastic band snapping or being moved, and jars were then secured with up to four strong elastic bands, which prevented escape, but did not prevent escape attempts. Also, *P. sericata* was the only species that entered diapause at the lower temperature of 15.8°C. Some specimens entered the pupal and adult stages normally, whereas other entered diapause in the prepupal stage and remained in this stage for several months. Some died, and some successfully pupated and emerged several months later. A very few specimens entered diapause at 23.3°C.

Table 5 shows the minimum and maximum time taken for *L. illustris* to reach each developmental stage at 21.2  $\pm$  0.04°C. Only one experiment was conducted, consisting of three colonies (one disturbed by physical examination, one visually assessed, and one in which the jar itself was not moved, to ensure that just lifting the jar and removing the lid for visual examination did not disturb the development). All developed at the same rate. Only one temperature regime was examined, as *L. illustris* proved difficult to raise in the laboratory, and produced only sterile eggs in the third generation.

Three species, *P. sericata*, *E. latifrons*, and *C. vicina* were raised outside, in naturally fluctuating temperatures. Three jars of each species were raised. Figure 1 shows the maximum and minimum temperatures during development and the minimum development times for each stage and species. Both *E. latifrons* and *C. vicina* successfully completed development under these conditions. However, *P. sericata* entered diapause 19.8 days after oviposition. Temperatures were relatively warm during the beginning of the experiment, with daily maximums ranging from 12.4 to 23°C, and daily minimums ranging from 10 to 16°C. The overall mean time of *P.*

TABLE 5—Development data for *Lucilia illustris* (mean  $\pm$  standard error,  $\alpha = 0.05$ ).

Stage	21.2 $\pm$ 0.04°C	
	Min	Max
1st instar	19.3 $\pm$ 1.7	32.5 $\pm$ 11.5
2nd instar	48	69
3rd instar (feeding stage)	93	93
Prepupal	162	162
Pupal stage	229.0 $\pm$ 17.3	286.0 $\pm$ 28.9
Adult	458.0 $\pm$ 8.0	573.3 $\pm$ 27.3

*sericata* development, until it entered diapause, was 15°C, similar to that under controlled conditions. As in lab conditions with only a slightly higher mean temperature, this species alone entered diapause despite highs reaching the low 20°Cs. Day length was shortening during these experiments. Time of eclosion for this species was the same under both lab conditions at 15.8°C and in outside conditions, although entry into each subsequent stage took slightly longer under fluctuating temperature conditions than under the relatively constant lab conditions. Also, only some specimens entered diapause at 15.8°C under lab conditions, whereas all specimens entered diapause in the fluctuating, outdoor temperatures.

*C. vicina* and *E. latifrons* continued to develop, despite consistently lowering temperatures, with highs of less than 13°C and lows of 6°C before all had emerged as adults. Development rates were considerably slowed, but continued to completion. At emergence, temperatures were frequently too low for the adults to fly; however, they had been able to emerge successfully.

Figure 2 shows the mean percent time (minimum) spent in each developmental stage (from beginning to end) for each species at each temperature. No data are given for *P. sericata* at fluctuating, outdoor temperatures as development was not completed. Table 6 shows the results of the analysis of variance, two-factor without replication, performed on the mean minimum percent time spent in each stage for all experiments conducted with that species. No evident interactions were seen. As can be seen, although Fig. 2 shows variation in the percent time spent in each stage, Table 6 indicates that there were no significant differences in minimum percent time spent in the egg, 1st instar, 2nd instar, or 3rd instar. Table 6 shows that there is a significant difference between species in the percent time spent in the prepupal stage (nonfeeding stage of the third instar) and the pupal stage.

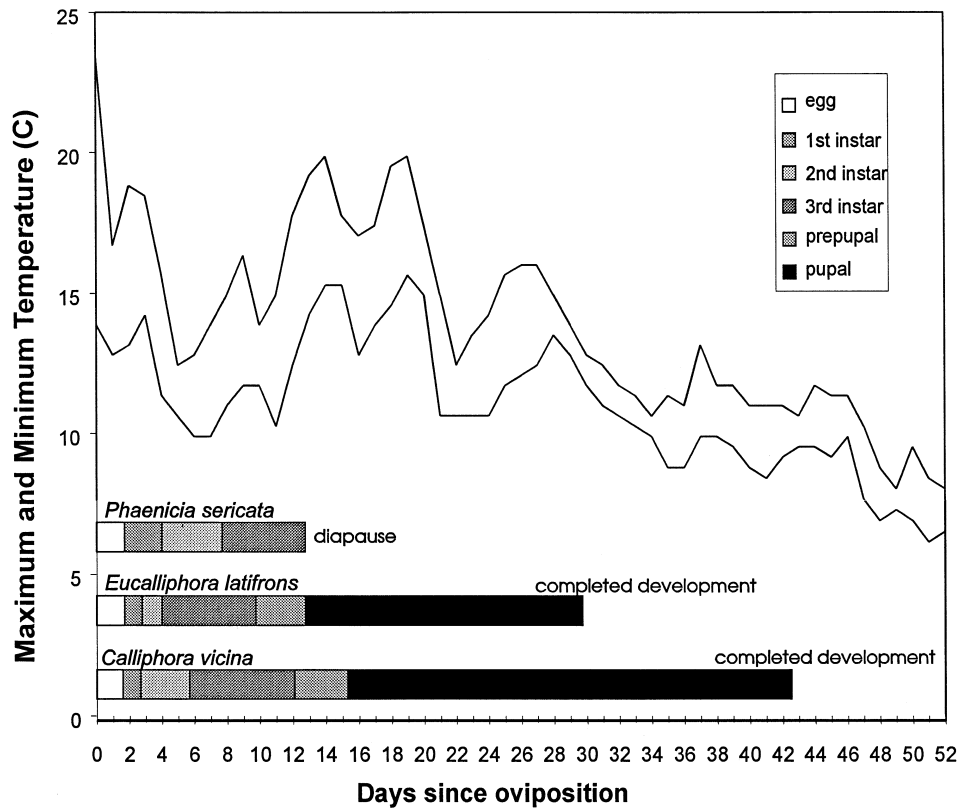


FIG. 1—Maximum and minimum temperatures recorded outside during experiment and minimum development times for each stage of three species of Calliphoridae, *Phaenicia sericata*, *Eucalliphora latifrons*, and *Calliphora vicina*.

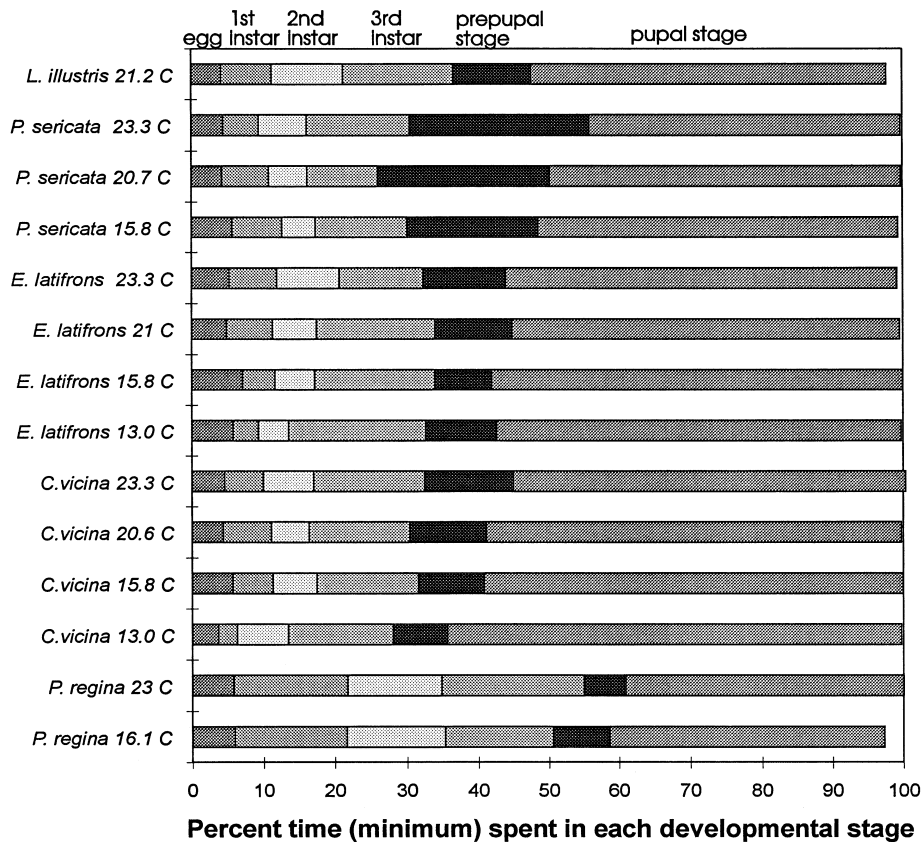


FIG. 2—Mean percent time (minimum) spent in each developmental stage (from beginning to end) for five blow fly species at several temperatures. No data are given for *Phaenicia sericata* during outside experiment as all insects entered diapause, so development was not completed.

Figure 3 shows the maximum and minimum overall length of the larvae in the 1st, 2nd, and 3rd larval stages, and the maximum length of the crop during these stages. No data are given for the prepupal stage, as handling of the insects when they began to contract appeared to disturb the insects in preliminary experiments. However, during this stage, they contracted, became opaque so that internal structures, such as mouth parts, were no longer visible to the naked eye, and the crop receded and the contents were eventually totally utilized prior to pupation.

TABLE 6—Results of analysis of variance (ANOVA), two factor, without replication ( $\alpha = 0.05$ ), on mean percent time (minimum) spent in each developmental stage for five Calliphoridae species, *Lucilia illustris*, *Phaenicia sericata*, *Eucalliphora latifrons*, *Calliphora vicina* and *Phormia regina*, under up to four temperature regimes, 15.8°C, 21.0°C, 23.3°C, and fluctuating, outdoor conditions. Data shown in Fig. 2.

Development Stage	Species		Temperature	
	F	P	F	P
Egg	3.191	0.053	2.043	0.162
1st instar	0.961	0.463	1.620	0.237
2nd instar	2.648	0.086	2.124	0.151
3rd instar	2.041	0.152	0.694	0.573
Prepupal	4.299	0.022	2.104	0.153
Pupal	5.118	0.012	1.062	0.401

**Discussion**

The minimal handling of the larvae used in these experiments appeared to have no effect on the insect’s behavior or development. We have previously shown that limited handling of a sample of the insects, such as used here, does not affect developmental rates (41). This is supported by Ash and Greenberg (27), who noted that handling did not delay development in *P. sericata*, although it did affect development in *P. pallescens* (Shannon), a subtropical species found in the Southern U.S. and not found in Canada. The only exception to this was noted in my preliminary experiments in which handling of the prepupal stage, just prior to pupation, delayed pupation slightly. Mackerras, in Australia, also noted that, in *P. (=Lucilia) sericata*, handling of the prepupal stage only delayed pupation by up to four days (42). Hence, prepupal larvae in these experiments were not handled, only visually observed, once it was determined that they had entered this stage.

Development was speeded up by higher temperatures, and complete egg-adult development took between 1.4 to 2.3 times longer at the lower temperatures. It is a well recognized fact that insect development is temperature dependent; that is, the normal metabolic rate is increased with increased temperature, which results in a faster rate of development, so that the duration of development decreases in a linear manner with increasing temperature within optimum ranges (Chapman 1980). This has meant that, in many forensic cases, data generated at a particular temperature are converted to thermal units, either accumulated degree days (ADD) or accumulated degree hours (ADH) (43–45,22). ADD or ADH are calcu-

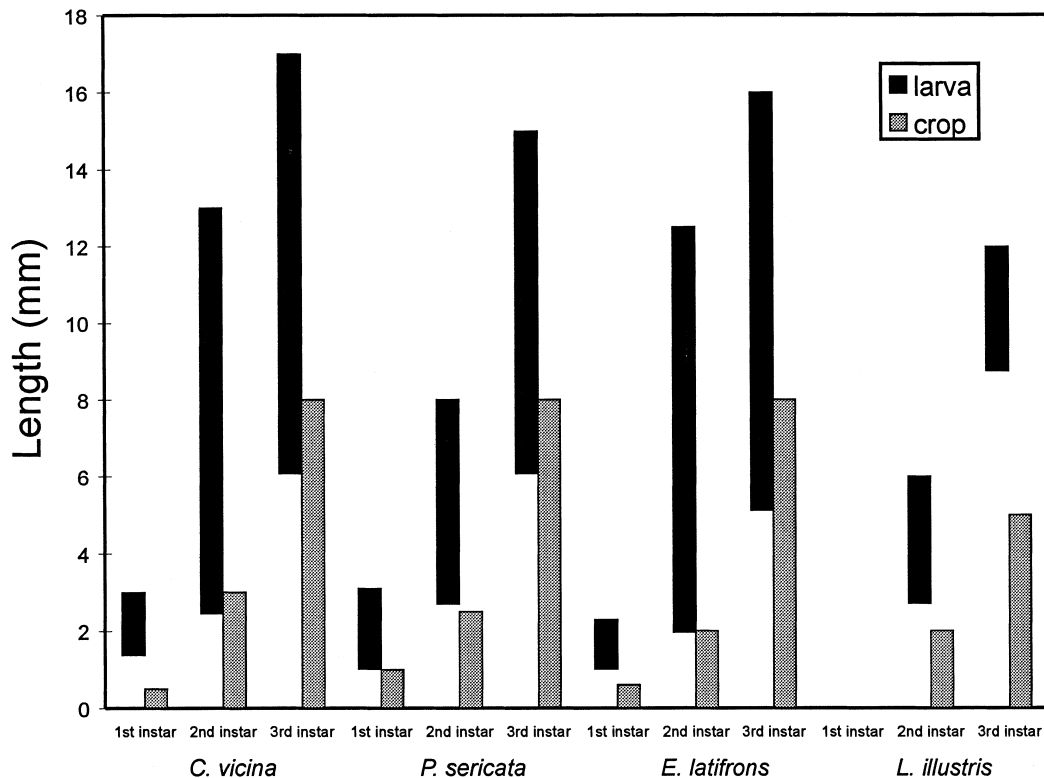


FIG. 3—Maximum and minimum lengths of whole larvae, and maximum and minimum lengths of crop for each species for 1st, 2nd, and 3rd instar larvae. No data are given for prepupal lengths, as handling ceased when all specimens had entered the prepupal stage to minimize the potential effects of handling.

lated by multiplying the developmental duration (in days or hours) for the time to reach a given stage, by the rearing temperature. This gives the number of ADD or ADH required to reach a given stage of development. The ADD or ADH for a given time period is calculated by a simple reverse summation process (43–45,22). This technique has been used in several published forensic analyses (43–45,22).

The use of ADD is based on the supposed linear relationship between temperature and development. However, this only appears to be valid when the data used to generate the degree days are close to the temperature at which the degree days will be applied. For instance, if development rates are needed for a temperature of 18°C, then degree days generated at a temperature of 16 to 20°C are probably valid. However, as the temperature gap becomes wider, it is noted that the relationship between temperature and development becomes less linear, particularly at the extremes of the optimum range. If development was entirely linear, then ADD should be the same for a species at any temperature. If ADD from egg to adulthood are determined for *P. regina* from data generated at 16.1°C, the total ADD would be 480.5 (from Table 1, calculated as described above). If the relationship between temperature and development was entirely linear, then the ADD should be the same or very close at 23°C, i.e., 480.5. However, the actual figure is 353.9. This is apparent in other data, (e.g., 46,22,27). However, when the gap between temperatures is much less, the linear principle holds true. For instance, at 15.8°C, *P. sericata* requires 510 ADD to reach adulthood, 419 ADD at 20.7°C, and 455 ADD at 23.3°C (from Table 4). This also clearly depends on the range used and the species. For example, in one study, *Phaenicia sericata* took a mean of 54.4 days to complete development at 19°C or 1033.6 ADD, 16 days at 27°C, or 431.5 ADD and 12.3 days at 35°C, or 430.5 ADD (27). From this it is clear that, although blow fly development is reasonably linear over temperature in most cases, care must be taken when using such data to generate very specific elapsed time since death estimations. It is clearly best to use data generated at a temperature as close as possible to that of the crime scene (43,44). Data have been generated for several blow fly species at different temperatures, (46,22,26–28), but the majority of these studies looked at development at temperatures much higher than those usually seen in British Columbia. The mean temperature at crime scenes in British Columbia is frequently 16 to 17°C (personal observations).

Earlier research conducted in the State of Washington on development rates of several blow fly species, including *Phormia regina*, *Phaenicia sericata*, and *Calliphora vicina* which are studied here, indicate much more rapid developmental times than were seen here (28). These earlier experiments were conducted at a single temperature, 26.6°C (80°F) which is several degrees warmer than that used in these experiments. Even so, it is clear that Kamal's data indicate much more rapid developmental times than are reported here. Kamal also indicates a more rapid developmental rate than has been recorded by other authors at similar temperatures (e.g., 22,27), whose data more closely relate to that presented here. These differences may be due to several factors. The present study and others cited (22,27), used incubators to maintain temperature control; however, Kamal did not have incubators available and used rooms in an insectary, which originally had little temperature control, and were later fitted with "an air conditioner" part way into the experiments (28). Consequently, temperature may have fluctuated greatly during Kamal's experiments, particularly in summer, resulting in faster development. As well, Kamal did not state times of examination, but the data indicates hourly examinations. As up to 29 generations were examined for any one species, it is unlikely

that the developing insects were checked hourly over this time period. Therefore, the precision of these data is difficult to evaluate. Finally, mean, mode, and maximum were given in Kamal's report (28), but the minimum times for each stage are much less than the sum of the entire developmental cycle, which is not explained.

All species, except *L. illustris*, adapted well to lab conditions and were easily raised through several generations. All left the beef liver as they entered the prepupal stage and moved through the sawdust before pupating. However, *P. sericata* was notable in its strong attempts to escape from the jar. This species invariably climbed the glass walls and was sometimes capable of pushing off the elastic band holding the paper toweling in place. This activity is still apparent in specimens from colonies that have been lab raised for over 25 years (personal observations). This species normally travels a considerable distance from its food source to pupate (22,5) so the excessive amount of movement in the jar is presumably a reflection of this desire to travel away from the food source.

An often asked question is whether lab data, generated at constant temperatures, are a reliable indicator of development rates under natural conditions, which fluctuate diurnally. In many insect species, developmental rates have been shown to be the same at natural, fluctuating temperatures, as at constant temperatures, when the temperature range is suitable, and the constant temperatures represent the mean of the fluctuating temperatures (47–52). Some insect species have been reported to develop more rapidly under fluctuating temperatures than under constant. These include some Coleoptera (50), some aphids (53,54) and milkweed bugs, *Oncopeltus fasciatus* (Dallas) (55). In some Hymenoptera, development may take slightly more or slightly less time in fluctuating conditions than at constant temperatures, depending on the temperatures studied (56), which probably depends on the number of accumulated thermal units.

In some cases, insects take longer to develop under fluctuating temperatures than under constant temperatures (57). In Calliphoridae, Greenberg (22) found that in the four species tested, *P. sericata*, *P. regina*, *Chrysomya rufifacies* (Macquart), and *Cochliomyia macellaria* (F.), development was slightly longer at fluctuating temperatures than at a mean, constant temperature by 20, 7, 9, and 12%, respectively. This was significant only in the case of *P. sericata*. Greenberg suggested that this retardation of development due to diurnal temperature changes should be taken into account. In Britain, *C. vicina* was also found to develop more slowly in a fluctuating temperature regime (58). However, in Australia, a related species, *Lucilia cuprina* (Wiedemann), the Australian sheep blow fly, was found to develop at the same rates at constant and fluctuating temperatures with the same mean at temperatures up to 30°C (59). Also, in British Columbia, comparisons with data obtained at constant temperatures for *P. regina* in these experiments compared well with those seen in carrion in the field at temperatures which fluctuated strongly diurnally, around the lab mean (30,33,60). In these experiments, *P. sericata* took very slightly longer to develop at fluctuating temperatures. As forensic entomologists usually estimate a minimum elapsed time since death, this means that any error generated by using data developed at constant temperatures will tend to be conservative.

There were no significant differences between percent time spent in the egg, 1st, 2nd, or 3rd instar, between the five species, or the four temperatures (Fig. 2 and Table 6). This is in agreement with previous work by Greenberg (22,61).

The size of a maggot has sometimes been used to determine age (62) and forensic entomologists are sometimes requested to age

maggots in photographs, without access to any actual specimens. As can be clearly seen here (Fig. 3), there is considerable overlap between length of maggot between stages, in particular between 2nd and 3rd instar, in most species. Therefore, size alone should not normally be used as the sole age determinant. This is confirmed by the accidental observations that starved larvae resulted in very small adult insects and, presumably, smaller larvae than nonstarved specimens. However, developmental rate was not affected. Therefore, if using length alone, starved specimens may be mistaken for younger insects. This would result in an underestimate of elapsed time since death. Although this may still be of value, it may be misleading to investigators. In carrion studies in British Columbia, undersized 2nd and 3rd instar Calliphoridae were observed leaving depleted pig carcasses (60,35). Also, although the lack of a visible crop in large larvae is usually an indication that an animal has entered the prepupal stage, this again must be checked physically, as the crop is often not visible in starved 3rd instar (personal observations). Therefore, although larval length may give an indication of age, it should not be used as the sole predictor.

#### Acknowledgments

The author thanks S. L. VanLaerhoven, L. M. Poirier, and H. LeBlanc for advice.

#### References

1. Busvine JR. Insects and hygiene. The biology and control of insect pests of medical and domestic importance. 3rd ed. London: Chapman and Hall, 1980.
2. Nuorteva P. Studies on the significance of flies in the transmission of poliomyelitis. *Ann Entomol Fenn* 1959;25:137–62.
3. French NP, Wall R, Morgan KL. The seasonal pattern of sheep blowfly strike in England and Wales. *Med Vet Entomol* 1995;9:1–8.
4. Wall R, French NP, Morgan KL. Population suppression for control of the blowfly *Lucilia sericata* and sheep blowfly strike. *Ecol Entomol* 1995;20:91–7.
5. Cragg JB. The natural history of sheep blowflies in Britain. *Ann Appl Biol* 1955;42:197–207.
6. Fotedar R, Banerjee U, Verma AK. Human cutaneous myiasis due to mixed infestation in a drug addict. *Ann Trop Med Parasitol* 1991; 85(3):339–40.
7. Hall RD, Anderson PC, Clark DP. A case of human myiasis caused by *Phormia regina* (Diptera: Calliphoridae) in Missouri, USA. *J Med Entomol* 1986;23(5):578–9.
8. Bauch R, Ziesenhenn K, Groskopf C. *Lucilia sericata* myiasis (Diptera: Calliphoridae) on a gangrene of the foot. *Angewandte Parasitologie* 1984;25(3):167–9.
9. Greenberg B. Two cases of human myiasis caused by *Phaenicia sericata* (Diptera: Calliphoridae) in Chicago area hospitals. *J Med Entomol* 1984;21:615.
10. James MT. The flies that cause myiasis in man. U.S. Dept. Agriculture, 1947.
11. Baer WS. The treatment of chronic osteomyelitis with the maggot (larva of the blow fly). *J Bone Joint Surgery* 1931;13:438–75.
12. Sherman RA, Wyle FA, Thrupp L. Effects of seven antibiotics on the growth and development of *Phaenicia sericata* (Diptera: Calliphoridae) larvae. *J Med Entomol* 1995;32(5):646–9.
13. Sherman RA, Wyle F, Vulpe M, Levson L, Castillo L. The utility of maggot therapy for treating pressure sores. *J Am Paraplegic Soc* 1993;16:269.
14. King AB, Flynn KJ. Maggot therapy revisited: a case study. *Dermatology Nursing* 1991;3:100–102.
15. Sherman RA, Pechter EA. Maggot therapy: a review of the therapeutic applications of fly larvae in human medicine, especially for treating osteomyelitis. *Med Vet Entomol* 1988;2(3):225–30.
16. Zotov VA, Grosse VWR, Treger B. Effect of photoperiod on diurnal rhythm of *Calliphora vicina* (Diptera: Calliphoridae). *Entomol Rev* 1991;70(1):102–5.
17. Currah L, Ockendon D. Pollination activity by blowflies and honeybees on onions in breeders cages. *Ann Appl Biol* 1984;105:167–76.
18. Smith BM, Jackson JC. The controlled pollination of seeding vegetable crops by means of blowflies. *Hort Res* 1976;10:53–5.
19. Anderson GS. The use of insects in death investigations: an analysis of forensic entomology cases in British Columbia over a five year period. *Can Soc Forensic Sci J* 1995;28(4):277–92.
20. Catts EP, Goff ML. Forensic entomology in criminal investigations. *Ann Rev Entomol* 1992;37:253–72.
21. Lord WD, Adkins TR, Catts EP. The use of *Synthesiomia nudesita* (Van Der Wulp) (Diptera: Muscidae) and *Calliphora vicina* (Robineau-Desvoidy) (Diptera: Calliphoridae) to estimate the time of death of a body buried under a house. *J Agric Entomol* 1992;9(4):227–35.
22. Greenberg B. Flies as forensic indicators. *J Med Entomol* 1991;28: 565–77.
23. Goff ML, Odom CB. Forensic entomology in the Hawaiian Islands, USA. Three case studies. *Am J For Med Path* 1987;8:45–50.
24. Lord WD, Catts EP, Scarboro DA, Hadfield DB. The green blowfly, *Lucilia illustris* (Meigen) as an indicator of human post-mortem interval: a case of homicide from Fort Lewis, Washington. *Bull Soc Vector Ecol* 1986;11(2):271–5.
25. Lord WD, Johnson RW, Johnson F. The blue bottle fly *Calliphora vicina* (= *erythrocephala*) as an indicator of human post-mortem interval: a case of homicide from suburban Washington DC. *Bull Soc Vector Ecol* 1986;11:276–80.
26. Nuorteva P. Sarcosaprophagous insects as forensic indicators. In *Forensic Medicine: A study in trauma and environmental hazards*, Tedeschi, CG, Eckert, WG, and Tedeschi, LG, editors. Philadelphia: W.B. Saunders Co., 1977.
27. Ash N, Greenberg B. Developmental temperature responses of the sibling species *Phaenicia sericata* and *Phaenicia pallescens*. *Ann Entomol Soc Am* 1975;68:197–200.
28. Kamal AS. Comparative study of thirteen species of sarcosaprophagous Calliphoridae and Sarcophagidae (Diptera) I. *Bionomics*. *Ann Entomol Soc Am* 1958;51:261–70.
29. VanLaerhoven SL, Anderson GS. Insect succession on buried carrion in two biogeoclimatic zones of British Columbia. *J Forensic Sci* 1999; 44:31–41.
30. Dillon LC. Insect succession on carrion in three biogeoclimatic zones in British Columbia. (M.Sc. thesis) Burnaby, B.C. Dept. of Biological Sciences, Simon Fraser University, 1997.
31. VanLaerhoven SL. Successional biodiversity in insect species on buried carrion in the Vancouver and Cariboo regions of British Columbia. (M.P.M. thesis) Burnaby, B.C. Dept. of Biological Sciences, Simon Fraser University, 1997.
32. Anderson GS, VanLaerhoven SL. Initial studies on insect succession on carrion in Southwestern British Columbia. *J Forensic Sci* 1996;41(4): 617–25.
33. Dillon LC, Anderson GS. Forensic entomology: a database for insect succession on carrion in Northern and Interior B.C. Canadian Police Research Centre, Ottawa, Ontario, 1996 Technical Report TR-04-96.
34. VanLaerhoven SL, Anderson GS. Forensic entomology. Determining time of death in buried homicide victims using insect succession. Canadian Police Research Centre, Ottawa, Ontario, 1996 Technical Report TR-02-96.
35. Dillon LC, Anderson GS. Forensic entomology: the use of insects in death investigations to determine elapsed time since death. Canadian Police Research Centre, Ottawa, Ontario, 1995 Technical Report TR-05-95.
36. Hall DG. Blowflies of North America. Lafayette, Indiana: The Thomas Say Foundation, 1948.
37. Smith KGV. A manual of forensic entomology. London: Trustees of The British Museum (Nat. Hist.) and Cornell University Press, 1986.
38. Tessmer JW, Meek CL. Dispersal and distribution of Calliphoridae (Diptera) immatures from animal carcasses in Southern Louisiana. *J Med Entomol* 1996;33(4):665–9.
39. Greenberg B. Behaviour of postfeeding larvae of some Calliphoridae and a Muscid (Diptera). *Ann Entomol Soc Am* 1990;83(6):1210–14.
40. Catts EP, Haskell NH. Entomology and death—a procedural guide. Clemson, SC: Joyce's Print Shop, 1990.
41. Johl HK, Anderson GS. Effects of chilling on development of the blow fly *Calliphora vicina* Robineau-Desvoidy (Diptera) and their relationship to time of death. *J Entomol Soc BC* 1996;93:93–8.
42. MacKerras MJ. Observations on the life-histories, nutritional requirements and fecundity of blowflies. *Bull Entomol Res* 1933;24:353–62.
43. Anderson GS. The use of insects to determine the time of decapitation: a case study from British Columbia. *J Forensic Sci* 1997;42(5):947–50.



44. Lord WD, Goff ML, Adkins TR, Haskell NH. The black soldier fly *Hermetia illucens* (Diptera: Stratiomyidae) as a potential measure of human postmortem interval: observations and case histories. *J Forensic Sci* 1994;39(1):215–22.
45. Goff ML, Charbonneau S, Sullivan W. Presence of fecal matter in diapers as potential source of error in estimations of postmortem intervals using arthropod development patterns. *J Forensic Sci* 1991;36(5):1603–6.
46. Greenberg B, Tantawi TI. Different developmental strategies in two boreal blow flies (Diptera: Calliphoridae). *J Med Entomol* 1993;30(2):481–4.
47. Butler GD, Lopez JD. *Trichogramma pretiosum*: development in relation to constant and fluctuating temperatures. *Ann Entomol Soc Am* 1980;73:671–3.
48. Neumann D, Heimbach F. Das Wachstum des Kohlweisslings bei konstanten und tagesperiodisch wechselnden Temperaturen. *Oecologia* 1975;20:135–41.
49. Welbers P. Der Einfluss von tagesperiodischen Wechseltemperaturen bei de Motte *Pectinophora*. I. Entwicklungsdauer, Larvengewicht, and Reproduktionsrate. *Oecologia* 1975;21:31–42.
50. Hagstrum DW, Leach CE. Role of constant and fluctuating temperatures in determining developmental time and fecundity of three species of stored products Coleoptera. *Ann Entomol Soc Am* 1972;66:407–10.
51. Champlain RA, Butler GD. Temperature effects on the development of the egg and nymphal stages of *Lygus hesperus* (Hemiptera: Miridae). *Ann Entomol Soc Am* 1967;60:519–21.
52. Matteson JW, Decker GC. Development of the European corn borer at controlled constant and variable temperatures. *J Econ Entomol* 1965;58:344–9.
53. Messenger PS. Bioclimatic studies of the aphid parasite *Praon exsolentum*. 2. Thermal limits to development and occurrence of diapause. *Ann Entomol Soc Am* 1969;62:1026–31.
54. Messenger PS. The influence of rhythmically fluctuating temperatures on the development and reproduction of the spotted alfalfa aphid, *Theoraphis maculata*. *J Econ Entomol* 1964;57:71–6.
55. Richards AG, Suanraksa S. Energy expenditure during embryonic development under constant versus variable temperatures (*Oncopeltus fasciatus* (Dallas)). *Entomol Exp Appl* 1962;5:167–78.
56. Yeargan KV. Effects of temperature on developmental rate of *Telenomus podisi* (Hymenoptera: Scelionidae). *Ann Entomol Soc Am* 1980;73:339–42.
57. Bradshaw WE. Thermoperiodism and the thermal environment of the pitcher-plant mosquito, *Wyeomyia smithii*. *Oecologia* 1980;46:13–7.
58. Erzinclioglu Z. Blowflies. *Naturalist's Handbooks*. Vol. 23. Slough: Richmond Publishing Co. Ltd., 1996.
59. Dallwitz R. The influence of constant and fluctuating temperatures on development and survival rate of pupae of the Australian sheep blowfly, *Lucilia cuprina*. *Entomol Exp Appl* 1984;36:89–95.
60. Dillon LC. Personal communication. Dept. of Biological Sciences, Simon Fraser University, presently Coroner, B.C. Coroners Service, New Westminster, BC.
61. Greenberg B, Szyska ML. Immature stages and biology of fifteen species of Peruvian Calliphoridae (Diptera). *Ann Entomol Soc Am* 1984;77:488–517.
62. Tantawi TI, Greenberg B. The effect of killing and preservative solutions on estimates of maggot age in forensic cases. *J Forensic Sci* 1993;38(3):702–7.

## Additional information and reprint requests:

Dr. Gail S. Anderson  
 School of Criminology  
 Simon Fraser University  
 8888 University Drive  
 Burnaby, B.C.  
 V5A 1S6  
 Canada