Implications of using development rates of blow fly (Diptera: Calliphoridae) eggs to determine postmortem interval

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ABSTRACT

This research examined the eclosion times of blow fly eggs to determine whether eggs begin to develop at the time of oviposition, or *in vivo*. Eggs were obtained from laboratory colonies of *Calliphora vicina* Robineau-Desvoidy, *Phaenicia sericata* (Meigen) and *Eucalliphora latifrons* (Hough) and observed at 2-h intervals. All three species had eggs eclose earlier than the expected minimum of 22 h at 21°C. Precocious egg development occurred for 75% of the *C. vicina* egg mass, while 100% of the *E. latifrons* and *P. sericata* egg masses developed early. Subsequently, we denied an oviposition medium to fresh *C. vicina* and *P. sericata* colonies for 7 and 14 d and compared the eclosion times with that of eggs from colonies with a continual access to beef liver. In both species, no precocious egg development was observed as the eggs eclosed 3-4 h after the expected minimum time of eclosion in both treatments and control. Finally, we examined eclosion times of eggs laid by blow flies in the wild. Eggs laid in the wild by *P. sericata* and *C. vicina* also took 1-3 h longer to eclose than the expected minimum time of eclosion. Our first experiment demonstrated that eggs laid by a single female at one time, can eclose at a wide variety of times, ranging from 2 h to the expected 22 h after oviposition at 21°C. Our inability to repeat the early eclosion in the laboratory with new colonies, despite the denial of oviposition media, or in the wild under natural conditions, is reassuring to those using egg development and eclosion to determine elapsed time since death. Clearly this phenomenon is not common, and may be explained as an artifact of laboratory colonies that do not have a regular influx of wild blow flies.

Key words: forensic entomology, medico-legal entomology, elapsed time since death

INTRODUCTION

Forensic entomology, or the use of insects to determine the elapsed time since death of a homicide victim, is a technique that has been employed in many homicide investigations worldwide (Goff 1992; Leclercq and Vaillant 1992; Lord *et al.* 1994; Anderson 1995). It is the most accurate and often the only method available to determine elapsed time since death after 72 h. However, it also is used during the first 72 h after death, particularly in high profile crimes, to confirm pathological parameters, or when only a portion of the body has been recovered. Traditionally, medical parameters are used to determine time since death in the first hours after death, but these involve many variables (Henssge *et al.* 1995) and pathologists are often reluctant to offer an opinion on time since death when more than

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a few hours have passed. Thirty percent of forensic entomology cases in Canada in 1995 involved blow fly (Diptera: Calliphoridae) egg evidence alone and this trend has continued (Anderson and Cervenka 2001). Although the cases were mainly homicide, they also included one poaching case where blow fly egg development evidence was vital in connecting time of death of bear cubs with the perpetrators at the scene (Anderson 1999).

Since blow flies usually arrive and begin laying eggs within minutes of death (Anderson and VanLaerhoven 1996), an analysis using the eclosion times of blow fly eggs will provide an estimate of the minimum time since death in the early postmortem interval. This method requires accurate research on the developmental rates of eggs. Previous research indicates that the time necessary for blow fly eggs to eclose depends on the species and the temperature (Kamal 1958; Nuorteva 1977; Greenberg 1993; Anderson 2000). However, these developmental rates and times of eclosion assume that egg development begins after oviposition and that the eggs do not begin to develop within the adult fly. Our laboratory research has indicated that this may not always be the case. If *in vivo* development does occur, this would change the estimate of elapsed time since death by as much as 24 h. We hypothesized that female flies which have a suitable oviposition medium available, will oviposit eggs which eclose after the normal length of time, after oviposition; whereas flies which are denied a suitable oviposition medium may have eggs developing *in vivo*, thereby decreasing the length of time between oviposition and eclosion.

The objectives of this research were to: determine whether insect eggs laid on a homicide victim begin to develop at the time of oviposition, or *in vivo*, as we have observed occasionally in the lab; and to determine whether early eclosion occurs in the wild or is an artifact of laboratory conditions.

MATERIALS AND METHODS

We examined egg eclosion under laboratory conditions at 21°C for three species of blow fly: *Calliphora vicina* Robineau-Desvoidy, *Phaenicia sericata* (Meigen) and *Eucalliphora latifrons* (Hough). All three species were reared in laboratory colonies descended from wild specimens collected locally in the Lower Mainland of British Columbia. They had been under laboratory conditions for approximately a year. On 5 March 1994, beef liver was presented to gravid females and after several hundred eggs were laid by ~10 females over a 30 min period, the liver was removed from the cages. Each egg mass was examined for eclosion immediately after oviposition and at 2 h intervals until eclosion.

The experiment was repeated at 21°C with new colonies of blow flies, this time varying the availability of an ovipositional medium. Fresh wild caught *C. vicina* and *P. sericata* colonies were established. On 1 May 1995, newly emerged adults of each species were exposed to fresh beef liver for 24 h to ensure that all received a protein meal for the development of ovaries and testes (Erzinclioglu 1996). The adults were then divided into three groups. The first group was given immediate and continuous access to beef liver as an oviposition medium. The second group was given only water and sugar for 7 d after the females were gravid, and was then given fresh beef liver as an oviposition medium. The final group was given water and sugar but was denied an oviposition medium until 14 d after the females were gravid. A minimum of 75 males and 75 females of each species were used, with one cage per species per treatment. Five females were dissected each day to determine the time taken until eggs were mature. The delayed access to an oviposition medium was timed after the females were gravid. After each group was presented with beef liver, and eggs were laid over a 30-min period, the egg mass was removed from the cage and observed every 2 h until all the eggs had eclosed.

We also tested eggs laid by blow fly females in the wild. Petri dishes with approximately 250 g of fresh beef liver were exposed in partially sunny locations in Coquitlam, BC. The experiments were conducted between 17-25 September 1996 and 2-10 June 1997. At all times, blow flies were abundant in this mild region. The experiment was replicated 15 times. After oviposition of at least 100 eggs in a 30-min period, the petri dishes were covered to prevent further oviposition and moved indoors. Each egg mass was examined for eclosion at 2-h intervals until eclosion.

Ambient temperature was recorded at 30-min intervals throughout each experiment using a double channel datalogger (SmartReader 1®, Young Environmental Systems, Richmond, BC). Temperatures cited are means of records from the time eggs were laid until eclosion was complete.

RESULTS

All three species had eggs eclose earlier than expected at 21°C (Table 1). Precocious egg development occurred for 75% of the *C. vicina* egg mass, while 100% of the *E. latifrons* and *P. sericata* egg masses developed early (Fig. 1).

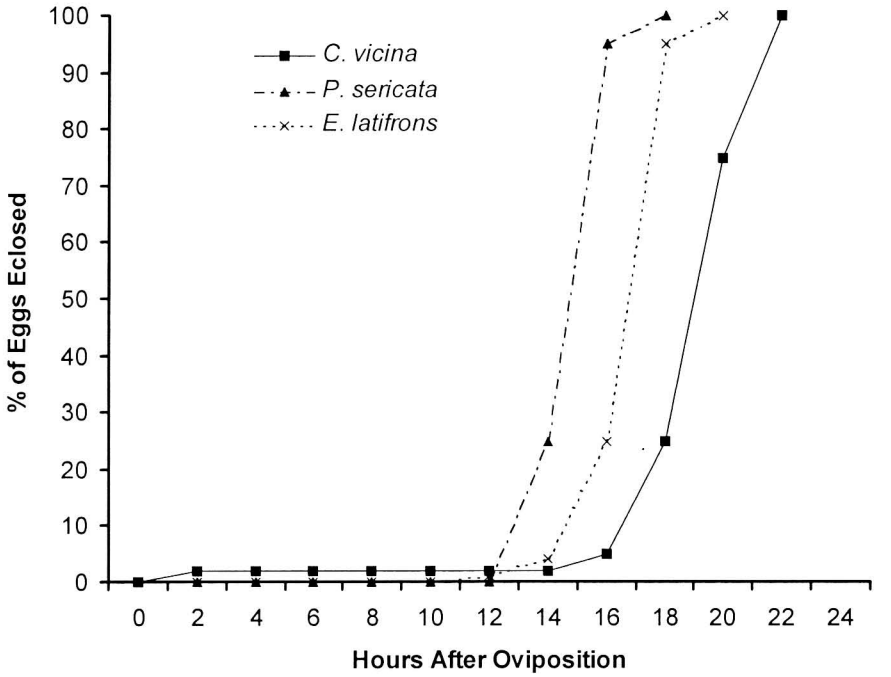

Figure 1. Percent of eggs eclosed from egg masses of three laboratory colonies of blow flies.

When new colonies of *P. sericata* and *C. vicina* were established, no precocious egg development was observed (Table 2), despite the lack of ovipositional media. *Phaenicia sericata* and *C. vicina* females took 3 d at 21°C to develop mature eggs in their ovaries.

In the field experiments, no precocious egg development was observed for eggs laid by *P. sericata* and *C. vicina* (Table 3). The mean temperature was 20°C for the September experiment and 23°C for the June experiment.

Table 1

Egg eclosion from egg masses of laboratory colonies of blow flies compared to expected minimum times of eclosion at 21°C (Anderson 2000).

	Minimum Time of Eclosion (h)	
Species	Expected	Observed
C. vicina	22	2
P. sericata	21	14
E. latifrons	22	12

Table 2

Egg eclosion from egg masses of laboratory colonies of blow flies: held continuously with oviposition media available; held 7 d without oviposition media; and held 14 d without oviposition media, compared to expected minimum times of eclosion at 21°C (Anderson 2000).

		Minimum Time of Eclosion (h)	
Oviposition media	Species	Expected	Observed
Available	*C. vicina*	22	26
Available	*P. sericata*	21	24
7 d	*C. vicina*	22	26
7 d	*P. sericata*	21	24
14 d	*C. vicina*	22	26
14 d	*P. sericata*	21	24

Table 3

Egg eclosion from egg masses of wild blow flies compared to expected minimum times of eclosion (Anderson 2000).

		Minimum Time of Eclosion (h)	
Mean Temperature	Species	Expected	Observed
20°C	*C. vicina*	26	28
20°C	*P. sericata*	25	26
23°C	*C. vicina*	21	24
23°C	*P. sericata*	21	24

DISCUSSION

It is currently accepted that blow fly eggs do not generally develop in the female fly, but only begin to develop after oviposition. Therefore, a measure of the developmental stage can be used to predict the age of the egg, and the time of eclosion can be used to count backwards to determine the time of oviposition. However, our first laboratory experiment demonstrated that eggs laid at the same time can eclose at a wide variety of times, ranging from 2 h to the expected 22 h after oviposition.

Early eclosion of blow fly eggs has been described in the literature, although it is rare (Auten 1934; Reiter 1984; Erzinclioglu 1990). It is possible that female flies may delay oviposition until a suitable site is found (Auten 1934). One recent study examined internal egg development of *Phormia regina* (Meigen) and stated that only one developing egg can be withheld by females, as this one egg enters the oviduct and is fertilized, whereas, the rest

do not enter the oviduct until oviposition (Erzinclioglu 1990). Another study examined *Calliphora terraenovae* Macquart, *C. vomitoria* (L.), *C. vicina* and *P. sericata* (Meigen) and found precocious egg development of at least one egg within all four species (Wells and King 2001).

The trigger for development within the female remains unknown. Our inability to repeat the early eclosion in the laboratory with new, wild-captured colonies, despite the denial of oviposition media, or in the wild under natural conditions, is reassuring to those using egg development and eclosion to determine elapsed time since death. Clearly this phenomenon is not common, and may be explained as an artifact of lab colonies that do not have a regular influx of wild blow flies; it may even have been an artifact of those specific colonies, although this seems unlikely. In fact, in a large number of other experiments conducted over several years, in which eggs were observed every 1-2 h until eclosion, not once was this phenomenon observed (Anderson 2000). As well, many other researchers who have performed similar experiments have not mentioned early eclosion (Melvin 1934; Kamal 1958; Nuorteva 1977; Nishida 1984; Greenberg 1993).

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