Effects of refrigeration on development of the blow fly, *Calliphora vicina* (Diptera: Calliphoridae) and their relationship to time of death

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ABSTRACT

Eggs, larvae, and pupae of the blow fly *Calliphora vicina* reared normally at 24°C were kept for 24 h at an ambient temperature of 3.0±0.14°C during growth and development. This was to simulate the chilling of insects before their collection from cadavers in a morgue at 3°C in forensic entomology cases. Such treatment of any stage induced a 24 h delay in adult emergence because the insects did not appear to develop while chilled. No mortality occurred in any stage, including eggs and 1st instar larvae, during chilling. This information is important for, and should improve the accuracy of forensic investigations.

Key words: Forensic entomology, maggot, British Columbia, refrigeration, development

INTRODUCTION

Forensic entomology which includes the study of the ecology and development of insects colonizing a corpse, is commonly used in investigations of death (Catts and Haskell 1990, Smith 1986). Analysis of the developmental stages of insects on the remains, together with environmental information, in particular temperature at the crime scene, can allow more precise estimates of time of death than are possible by any other means when death occurred at least 3 days before discovery (Sperling *et al.* 1994; Kashyap and Pillai 1989). Ideally, insects should be collected directly from the remains, at the death scene, by the entomologist. But, this is not always possible, and the corpse is often moved to a morgue and chilled before an entomologist can examine it.

The objectives of this work were: to determine whether larvae continue to grow and develop when maintained at 3°C for 24 h periods, or whether development is arrested; if development is arrested, whether the chilled insects complete development normally when returned to a warmer environment; or whether there is a delay which persists during the rest of the development. These points are important in determining the age of the insects when they were collected and therefore, for estimating time of death of the corpse.

METHODS AND MATERIALS

Calliphora vicina Robineau-Desvoidy, a common blow fly found in many forensic entomology cases in B.C. (Anderson 1995) was used in this study. All the flies were from a laboratory colony established from individuals trapped in Burnaby, B.C.

Adult flies were allowed to oviposit on the surface of fresh beef liver. The colony was observed regularly and the liver removed when 1800-2000 eggs were obtained. Seven pairs of 4 litre, wide-mouthed glass jars were set up with 3 cm of moistened sawdust in each, to allow prepupae to burrow. About 100 gm of beef liver were placed in each jar

on paper towels, to prevent newly emerged larvae from drowning. Approximately 100 eggs were placed on the liver in each jar and the jars sealed with two layers of paper toweling secured with elastic bands. The jars were held at a mean temperature of 24.1°C, (monitored in both laboratory and incubator using SmartReader Dataloggers[1] set to record the means of 30 min. temperature readings over 30 days).

The first pair of jars was used as an overall control and kept at 24°C for the entire experiment. One of each of the remaining 6 pairs of jars of larvae was not disturbed until the adults had emerged, whereas the second jar was examined daily. Twenty larvae were randomly selected and removed from this jar daily for brief examination under a dissecting microscope and then replaced. When all 20 larvae examined were at a particular developmental stage the jar was chilled for 24 hours. The developmental stage was determined from their spiracular slits (Fig. 1) and crop size (Fig. 2). First instar larvae have no sclerotization around their spiracular slits (Fig. 1a) and their crops are not visible to the naked eye. Second instar larvae have sclerotization around their 2 pairs of spiracular slits visible under a dissecting microscope (Fig. 1b). During this stage the crop is still not visible to the naked eye. Third instar larvae have sclerotization around all 3 pairs of spiracular slits (Fig. 1c).

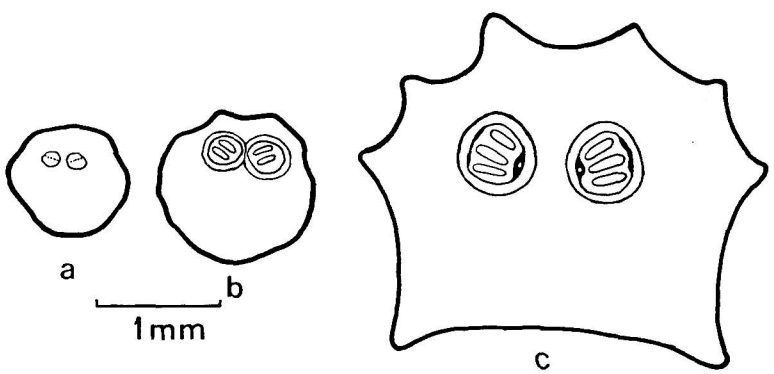

Figure 1. Diagrams of spiracles at posterior of *Calliphora vicina*. a) - First instar, cuticle not sclerotized, b) - Second instar, two pairs of spiracular slits clearly visible, c) - Third instar, three pairs of slits.

Their crops are visible to the naked eye (Fig. 2a) and during this stage the larvae feed voraciously. Then in the latter part of the third instar, they enter a prepupal or non-feeding stage. They leave the food source and scatter throughout the sawdust. They enter this stage with a full crop, but its contents gradually decrease during the prepupal stage (Fig. 2b). The crop shrinks until it can no longer be seen with the dissecting microscope (Fig. 2c) and the larvae contract to about half the length they were at the begining of the third instar. The entire process of crop reduction can be easily monitored (Fig. 2 a-c). Once all the insects in a given jar reached the third instar, all examinations were from the outside only, to avoid further handling. Experimental pairs 2-7, each consisting of an undisturbed and an examined jar, were chilled for 24 hours at a constant morgue temperature of 3.0±0.14°C (30 day mean ± SE) at one of the following developmental stages: pair 2, egg stage; pair 3, 1st instar; pair 4, 2nd instar; pair 5, 3rd instar; pair 6,

[1] Young Environmental Systems, Richmond, BC

prepupal stage and pair 7, pupal stage. The insects were chilled as soon as they entered the stage. Time taken to reach each stage was monitored daily for the 20 insects from all examined jars, and time taken for complete development was monitored in the undisturbed jars. The time taken to reach any one stage of development was the mean of 20 measurements of the total number of hours from the time of oviposition.

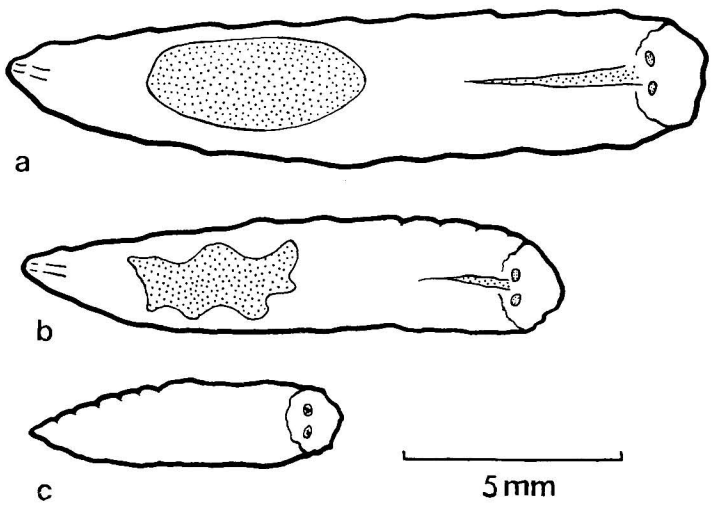

Figure 2. Diagrams of *Calliphora vicina* larvae during a) - early third instar, b) - late third instar, crop shrinking. c) - prepupal stage of third instar, crop invisible.

To observe development of the prepupa, a second experiment was carried out. An eighth pair of jars was set up and treated identically as for pairs 2-7. Insects in one jar were examined daily and in the other were not disturbed, except that at the beginning of the prepupal stage, when the crop was just beginning to decrease in size, the insects in both disturbed and nondisturbed jars were inspected, and 20 specimens at the same stage of development and crop reduction, were selected from each jar. The selected specimens were then placed in two new jars with fresh sawdust. One jar was chilled to 3°C for 24 h and the control jar was kept in the laboratory at room temperature. After chilling, all specimens were examined. The crop size of each of the chilled specimens was determined and compared with those in the control jar. Both jars were monitored later to determine time of adult emergence. No further handling was necessary.

RESULTS

The results of chilling *Calliphora vicina* at various developmental stages are summarized in Table 1. In all cases, larvae chilled for 24 h at some point in their development lagged behind controls in growth and development by 24 h. When chilled before the prepupal stage, there was a similar lag up to that stage. During this period, growth and development was delayed from 1 to 1.51 days compared with the controls. Chilled eggs and larvae reached the prepupal state at the same time as the controls but pupated 24h

later. Specimen chilled as prepupae or pupae, like those chilled as eggs and larvae emerged as adults 24h later than controls

Table 1

Effect of 24 hours of chilling on the duration of development in days of *Calliphora vicina* (**f**) crop full, (**r**) crop reduced, (**C**) stage chilled. Bold numbers indicate delays.

	Days to reach each stage						
Stage	control	eggs	1st	2nd	3rd	prepup.	pupa
Eggs	-	**(C)**	-	-	-	-	-
1st instar	1.00	**2.00**	1.00**(C)**	1.00	1.20	1.20	1.20
2nd instar	2.00	**3.51**	**3.52**	1.99**(C)**	2.01	2.01	2.02
3rd instar **(f)**	3.99	**4.96**	**4.96**	4.00	3.96**(C)**	3.96	4.20
3rd instar **(r)**	4.96	**6.30**	**6.30**	**6.30**	**6.30**	4.96	4.96
Prepupa	6.99	7.00	7.00	7.00	7.00	6.98**(C)**	7.01
Pupa	8.10	**8.99**	**8.99**	**8.99**	**8.99**	**9.00**	8.11**(C)**
Adult emerges	18.10	**19.20**	**19.20**	**19.20**	**19.20**	**19.20**	**19.20**

However, larvae treated in the 2nd instar showed no lag in growth and development until they reached the end of the feeding stage of the third instar. Handling the insects made no difference in their rate of development or time of emergence.

In the second experiment, (Table 2) the larvae were chilled just as they were entering the prepupal stage of the third instar when their crops began to shrink. The insects in both treated and control groups were examined immediately after chilling.

Table 2

Effect in days of 24 hours of chilling on late 3rd instar larvae with reduced crops, (**f**) crop full, (**r**) crop reduced, (**C**) stage chilled. Bold numbers indicate delays.

	Days to reach each stage		
Stage	unhandled, not chilled	handled, not chilled	handled & chilled
Eggs	-	-	-
1st instar	1.00	1.20	1.20
2nd instar	2.00	2.01	2.01
3rd instar **(f)**	3.99	4.20	4.20
3rd instar **(r)**	4.96	4.96	4.96**(C)**
Prepupa	6.99	6.40	6.40
Pupa	8.10	8.10	**9.00**
Adult emerges	18.10	17.11	**19.20**

Those that had been chilled had not developed any further and the crops were the same size as they were before treatment. However, the control insects kept at room temperature continued to develop and their crops were no longer visible. Clearly, chilling had arrested development. When these non-feeding 3rd instar larvae were monitored after chilling, there was an initial increase in the rate of development, and the treated larvae entered the prepupal stage 0.5 days earlier than the overall control (Pair 1). However, the rate of development of the treated group then slowed, and they pupated 0.9 days after the Pair 1 control group. This lag continued through the pupal stage and the adults emerged

1.1 days later. The untreated group also reached the prepupal state 0.5 days before the Pair 1 controls, then slowed down to reach the pupal state at the same time as the Pair 1 controls. However, this group emerged as adults a full day earlier than Pair 1 controls (Table 2).

DISCUSSION

Chilling at any immature stage for 24 h delayed adult emergence by 24 h and no measurable development occurred during the process. Clearly, chilling at 3°C arrested development, which resumed when the insects were returned to room temperature. A time lag equivalent to the time spent under chilling, was evident throughout development up to adult emergence.

The only exception to the above results occurred immediately after chilling in the second instar. In this case, no delay was seen until the insects entered the prepupal stage of the third instar. It is possible that this lack of effect immediately after chilling in the second instar was an anomalous one, because it occurred only at this one stage. All the insects were chilled as soon as they entered a particular stage, but, because there were gaps between examination times, it is possible that these insects had already been in the 2nd instar for several hours. This is a short stage and if moulting had already been initiated before chilling, it might have resumed immediately after the insects warmed up.

In all other cases where the insects were chilled before the prepupal stage, they lagged by 24 h until they were in the third instar. They were delayed entering the third instar by 24 h, then appeared to develop faster and entered the prepupal stage at the same time as the controls. However, development was again delayed 24 h during the pupal stage, and the flies emerged 24 h later than the controls. The fact that all specimens treated before the 3rd instar reacted in this manner indicated that it was not an experimental artifact. The reasons for this behaviour are speculative, and further work is needed to determine whether chilling has some physiological effect which is expressed only during this stage.

The minimal handling used in these experiments did not effect development rate or behaviour. This was also found by Ash and Greenberg (1975) who handled *Phaenicia sericata* (Meigen). Mackerras (1933) in Australia also noted that handling of all stages up to the prepupal stage had no effect on development. However, handling of the prepupal stage resulted in delayed pupation.

As we found development delayed equally in both disturbed and non-disturbed specimens, the effects we saw were clearly a result of the chilling and not the handling.

In the second experiment, 20 insects from each jar, one group disturbed daily, one not disturbed, were separated from the main group to see whether further reduction of the crop and, therefore, development had occurred. Although no development occurred during chilling and an overall 24 h lag in emergence time occured in chilled individuals, those specimens which were not chilled but were placed in a new jar spent less time in the pupal stage than any others and emerged a full day earlier than the control insects. This may have been due to handling, if the larvae are more susceptible to the same amount of handling at this stage than at earlier stages; but that seems unlikely, because handling had no effect at earlier stages, and handled, chilled larvae were affected in the same manner as those in the first experiment.

These specimens were separated from the rest of their group just as they entered the prepupal stage, when, in the wild, specimens wander from the food source to pupate (Smith 1986). It is possible that the low numbers of insects in this experiment resulted in few insect-to-insect encounters as they wandered through the sawdust, which may have contributed to more rapid pupation and emergence. Crowding can have indirect effects on

development in some species (Danks 1987). However, 100 insects/4 litre jar resulted in much less crowding than is usually seen on carrion in a wild situation (personal observations, GSA) so if the rapid development of this group is due to reduced numbers of insects, this is an experimental artifact, and such a situation is unlikely to occur in the wild.

These results have implications for forensic entomology. Determination of time since death is based on several factors, such as temperature, most importantly but also on the developmental stage of the immature insects collected. If the insects have reached a particular stage, for instance third instar, then, knowing recent local weather conditions, the age of these insects can be estimated. However, if the remains have been chilled overnight, and the larvae are in the third instar, they may have been in this stage when the remains were discovered the previous day, or may have continued to develop while chilled. We show here that there is no appreciable development during 24 h chilling. Recent work has shown that *Calliphora vicina* can continue to develop at an extremely low rate, under very cool conditions (103-115 days spent in the pupal stage at 5°C) (Davies and Ratcliffe 1994), but we found that chilling so slows down development as to be forensically insignificant. The delay in development approximates the chilled time.

Caution must be used when interpreting these results, because the chilled specimens in our experiments were in relatively low numbers and so formed only small masses. In such situations, cooling would rapidly affect all the insects in the jar. This would be an equivalent situation to that created by insects collected at the scene by investigators and chilled in small vials with the remains, which often occurs. Similarly, when remains are colonized by low numbers of insects, or when only partial remains are recovered, or when large numbers of insects are present, but are scattered rather than in masses, the insects would probably be affected more rapidly by the cooler temperatures. However, when the remains are colonized by large numbers of larvae, maggot masses are frequently present, raising the temperature of the corpse considerably above ambient. When such corpses are refrigerated it takes several hours before the entire remains are chilled, and in these cases, maggots usually continue to develop, particularly in the first hours of refrigeration (Catts 1992). In one human forensic case, the temperature within a mass of maggots was 20°C even after the remains had been refrigerated for four hours (GSA unpublished observations).

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