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# DEVELOPMENT OF *CALLIPHORA VICINA* ROBINEAU-DESVOIDY (DIPTERA, CALLIPHORIDAE) UNDER STARVATION CONDITIONS

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**Abstract:** An experiment designed for *Calliphora vicina* Robineau – Desvoidy, 1830 (Diptera, Calliphoridae) to check the potential pupariation capability of these flies when food is no longer available has been performed under laboratory conditions. Successful development has been obtained for maggots recently moulted into 3d instar but already feeding, with small but fertile adults being obtained that developed normally under laboratory conditions. The results of this experiment may be of great interest for specific cases when the corpse is removed from the scene but maggots remain there, and for archaeological research concerned with mummification processes.
**Key words:** Diptera, Calliphoridae, *Calliphora vicina*, forensic entomology, archaeology, starvation, development.

**Desarrollo de *Calliphora vicina* (Diptera, Calliphoridae) bajo condiciones de ayuno**
**Resumen:** Se ha diseñado un experimento para comprobar el potencial de pupación de *Calliphora vicina* Robineau – Desvoidy, 1830 (Diptera, Calliphoridae), en situaciones donde la fuente de comida se agota o deja de estar disponible. Se han obtenido desarrollos completos en aquellas larvas que han alcanzado la tercera fase o instar, obteniendo tanto pupas como adultos de pequeño tamaño pero fértiles, con desarrollo de una nueva población a partir de dichos adultos. Los resultados obtenidos de este estudio pueden ser de gran interés en casos específicos en los cuales el cuerpo haya sido trasladado del escenario del crimen, encontrando en su lugar unas pocas larvas remanentes. También pueden ser de gran ayuda para investigaciones arqueológicas relacionadas con procesos de momificación.
**Palabras clave:** Diptera, Calliphoridae, *Calliphora vicina,* entomología forense, arqueología, ayuno, desarrollo.

## Introduction

Necrophagous flies are useful in post-mortem interval estimations as they usually colonise a corpse within few minutes or hours after death, and develop in few days depending on the ambient temperature and food resources. Several experiments have been performed under controlled conditions to estimate the minimum time of development related to ambient temperature and food sources (Reiter, 1984; Saunders, 1997; Grassberger & Reiter, 2001, 2002a, 2002b; Kaneshrajah & Turner, 2004; Donovan *et al.*, 2006). As insect development depends on environmental temperature, it is usually expressed as Accumulated Degree Days (ADD) or Accumulated Degree Hours (ADH), depending whether the estimation of energy accumulated is based on daily or hourly temperature records (*cf.* Highley & Haskell, 2001). Such estimates are usually made with maggots fed *ad libitum* (*cf.* Reiter, 1984; Grassberger & Reiter, 2001, 2002a, 2002b; Kaneshrajah & Turner, 2004, Donovan *et al.*, 2006) until they arrive at their maximum length and migrate in search of an adequate substrate for pupariation. Experiments simulating extreme conditions (low temperature and absolute darkness D:D) shows induction to pupariation in larvae isolated from food source on day D4; after that (D5 and followings) low temperatures and darkness usually induced larvae into diapause (Saunders, 1997).

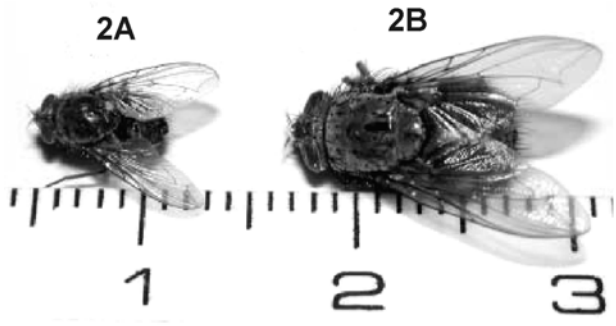
A question recently posed by an archaeologist through our e-mail list induced us to design a new experiment to check the potential pupariation capability of these flies when food is no longer available in environmental conditions closer to Ancient Egypt temples. We usually assume that larvae will die if no other food supply is found (Christopherson & Gibo, 1997); but, does this really happen under natural conditions? If environmental conditions are adequate and no more food can be found or reached, could maggots at any instars pupariate and develop into adults? If not, how long can they survive under starvation? And, last but not least, are those adults adequately developed and can they produce new generations?

The results of this experiment may be of forensic interest in cases where the corpse is intentionally removed from the crime scene (Wells *et al*., 2001), or the maggot mass overstretch the food resource, the soft tissues are gone before they reach the postfeeding stage and no other food source is colonised for a complete development.

## Material and methods

Adults of *Calliphora vicina* were used for this experiment. This is the first time that ADD estimations are made by forcing specimens to pupariation under starvation conditions and under a natural photoperiod 9:15 Light/Darkness (L/D) cycle, with mean laboratory temperature of 24.6 ºC (22.2 to 25.6ºC) in Autumn (Table I) and Spring (Table II) replicates of the experiment.

We focused this first research on this species as it is the most frequent in our geographical area, it can be collected all year round, it is dominant throughout the year except in summer, when greenbottle flies are also collected, and it is often mentioned in forensic cases and applied research done all over the world (Nuorteva *et al*., 1967; Lord, 1986; Benecke, 1998; Marchenko, 2001; Schroeder *et al*.,

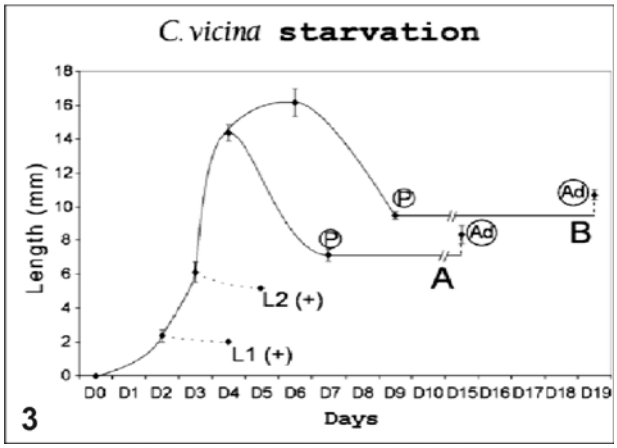




**Fig.1.** *Calliphora vicina*. Pupae normally developed from maggots fed *ad libitum* (B) and those from larvae isolated from the food source at the beginning of the third instar on D5 (A).

**Fig. 2.** *Calliphora vicina*. Adults normally developed from maggots fed *ad libitum* (B) and those from larvae isolated from the food source at the beginning of the third instar on D5 (A).

**Fig. 3.** *Calliphora vicina* development with A and B larvae. L1= first instar. L2 = second instar. P = pupae. Ad = adults.

2002; Arnaldos *et al*., 2005). Furthermore, this species has also been recorded in recent research done in Egypt (Tantawi *et al*., 1996), where it is considered a primary species as first coloniser of corpses together with *Lucilia sericata*. Therefore, we can expect the presence of *C. vicina* in ancient Egypt to be an important coloniser of corpses that were waiting to be mummified in the temples. We have also selected this species because it is easily cultured in laboratory conditions.

Attraction traps baited with pig kidney were placed in a suburban area on the campus of the University of the Basque Country (Leioa, Vizcaya, Spain). Small portions of viscera were placed in double funnel attraction traps (Turner) for long enough to allow the first necrophagous flies to arrive. Females were isolated from the traps and placed in rearing chambers to force oviposition under controlled conditions. After eclosion, larvae were allowed to feed for at least 24 hours. After that (1st day expressed as D1), subsamples of maggots were isolated each day from the food resource and placed under starvation conditions to induce pupariation. A second subsample was daily boiled in water and preserved in 80% ETOH for biometric analysis and alive specimens kept in plastic containers with different substrates made of tissue paper or soil to check for the possible influence of the substrate in the process.

## Results

A total of 271 individuals were used in two replicates of the experiment done in October 2005 (n=71) and May 2006 (n=200) (Tables 1 and 2). First (n=58) and second instar (n=54) removed prematurely from their food supply never succeed in pupating and died after two days of starvation.

Groups of first instar larvae (50) and second instar larvae (50) isolated from food were removed to see if they were able of pupate (Table II). However, none of them survived. This observation confirms that larvae are only able to pupate when they reach their last instar; further development is unsuccessful.

We finally detected no problems when larvae arrive to third instar. This could be due to the fact that larvae have taken in enough food to accumulate the minimum amount of energy needed (ADD 62.2-144.9 on base 10) to develop their internal organs fully and reach the last phase of their development with absolute success. In fact, all the larvae extracted in the third instar reached pupariation and developed into adults, including one moulted into L3 during day D4 (Table I).

We observed a big difference in size between puparia and adults developed from isolated larvae and those fed *ad libitum* (Table I, II). Early removal of maggots results in a clear decrease in size. Differences can reach 30% in length, ranging from a minimum of 6.4 mm obtained on D4 for pupariation and a maximum of 10 mm. obtained on D7 (fed *ad libitum*). Emergence of adults occurred 5 to 7 days after pupariation at a mean laboratory temperature of 22-25,4 ºC (Fig.1). No diapause is observed during winter months although L:D cycle is naturally reduced to 9:15 (Euskalmet, 2006), as no artificial regulation of L:D cycle is done for cultures. High laboratory temperatures may be the reason that explains the absence of diapause observed for this species.

The minimum size of puparia obtained depends on the day when larvae were removed. Minimum levels are obtained from the youngest maggots; those removed first (D4, D5; ADD(-B10): 74.6-160.3) developed into pupae and adults of 6.4 mm in length. The pupae size increases with

**Table I. *Calliphora vicina* reared under starvation conditions on October 2005. Report of successfully developed specimens, dimensions (mm), day of pupariation, emergence of adults and accumulated degree days (ADD).**

| Day | | Mean Tem (ºC) | Time of starvation | Instar | Pu-pae | Dead | Puparia (mm) | Emer-ged | Adult Emergence | Mean Tem (º C) | ADD (-B10) | Adults (mm) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| D0 | 19/10/2005 | 24.3 | - | - | - | - | - | - | - | | - | - |
| D1 | 20/10/2005 | 23.7 | - | - | - | - | - | - | - | | - | - |
| D2 | 21/10/2005 | 23.5 | 16:00 | L1 | 0 | 4 | - | - | - | | - | - |
| D2 | 21/10/2005 | 23.5 | 19:00 | L1 | 0 | 4 | - | - | - | | - | - |
| D3 | 23/10/2005 | 22.9 | 14:00 | L2 | 1 | 3 | - | - | - | | - | - |
| D3 | 23/10/2005 | 24.4 | 0:30 | L3 | 3 | 2 | 6,4 - 7 | 1 | D10 | 25.4 | 144.9 | 6,4 |
| D5 | 25/10/2005 | 25.6 | 13:30 | L3 | 4 | 4 | 6,6 - 7,6 | 4 | D11 | 25.5 | 160.3 | 7,8 - 9 |
| D5 | 25/10/2005 | 25.6 | 15:00 | L3 | 6 | 3 | 6,5 - 7,6 | 6 | D11 | 25.5 | 160.3 | 7,3 - 9 |
| D5 | 25/10/2005 | 25.6 | 16:15 | L3 | 7 | 3 | 6,3 - 8,2 | 7 | D11 | 25.5 | 160.3 | 7,7 - 8,9 |
| D5 | 25/10/2005 | 25.6 | 17:50 | L3 | 5 | 0 | 7 - 8,1 | 5 | D11 | 25.5 | 160.3 | 8,7 - 9,5 |
| D6 | 26/10/2005 | 24.6 | 11:00 | L3 | 5 | 0 | 7 - 8,6 | 5 | D11 | 25.5 | 160.3 | 8,1 - 11 |
| D6 | 26/10/2005 | 24.6 | 14:00 | L3 | 6 | 0 | 6,1 - 8 | 5 | D11 | 25.5 | 160.3 | 6,7 - 9,7 |
| D6 | 26/10/2005 | 24.6 | 17:45 | L3 | 7 | 0 | 6,1 - 8,9 | 5 | D11 | 25.5 | 160.3 | 8,2 -10,3 |
| D7 | 27/10/2005 | 25.3 | 11:21 | L3 | 2 | 0 | 8,2 - 9,7 | 2 | D14 | 24.9 | 205.3 | 9,8 - 11 |
| D7 | 27/10/2005 | 25.4 | 16:21 | L3 | 2 | 0 | 7,4 - 10 | 2 | D14 | 24.9 | 205.3 | 9,2 -11,2 |

**Table II. *Calliphora vicina* reared under starvation conditions on May 2006. Report of successfully developed specimens, dimensions (mm), day of pupariation, emergence of adults and accumulated degree days (ADD).**

| | Day | Mean Tem(º C) | ADD (-B10) | Instar | Starvation | Pupae | Dead | Larvae | | Puparia | | Adults | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Length | Width | Length | Width | Length | Width |
| D0 | 08/05/2006 | 22,2 | 12,2 | W | - | - | - | | | | | | |
| D1 | 09/05/2006 | 22,5 | 24,7 | L1 | - | - | - | | | | | | |
| D2 | 10/05/2006 | 22 | 36,7 | L1 | √ | 0 | 50 | 2,41 - 2,33 | 0,51 - 0,42 | (+) | (+) | (+) | (+) |
| D3 | 11/05/2006 | 22,9 | 49,6 | L2 | √ | 0 | 50 | 7,13 - 5,20 | 1,21 - 0,76 | (+) | (+) | (+) | (+) |
| D4 | 12/05/2006 | 22,6 | 62,2 | L3 | √ | | | 15,23 - 13,80 | 3,38 - 2,59 | | | | |
| D5 | 13/05/2006 | 22,4 | 74,6 | Pf - L3 | AL | | | | | | | | |
| D6 | 14/05/2006 | 23,6 | 88,2 | Pf - L3 | AL | | | | | | | | |
| D7 | 15/05/2006 | 24,3 | 102,5 | P - Pf | | 50 | 0 | 17,13 - 15 | 3,72 - 3,24 | 7,97 - 6,25 | 3,45 - 2,51 | | |
| D8 | 16/05/2006 | 24,5 | 117 | P - Pf | | | | | | | | | |
| D9 | 17/05/2006 | 24,9 | 131,9 | P - P | | 50 | 0 | | | 10 - 8,92 | 4,37 - 3,8 | | |
| D10 | 18/05/2006 | 24,3 | 146,2 | P - P | | | | | | | | | |
| D11 | 19/05/2006 | 24,3 | 160,5 | P - P | | | | | | | | | |
| D12 | 20/05/2006 | 24,4 | 174,9 | P - P | | | | | | | | | |
| D13 | 21/05/2006 | 24,6 | 189,5 | P - P | | | | | | | | | |
| D14 | 22/05/2006 | 24,9 | 204,4 | P - P | | | | | | | | | |
| D15 | 23/05/2006 | 24,2 | 218,6 | P - P | | | | | | | | | |
| D16 | 24/05/2006 | 24,2 | 232,8 | A - P | | | Adult | | | | | 9,22 - 7,23 | 3,58 - 2,43 |
| D17 | 25/05/2006 | 24,4 | 247,2 | P | | | | | | | | | |
| D18 | 26/05/2006 | 24,3 | 261,5 | P | | | | | | | | | |
| D19 | 27/05/2006 | 24,6 | 276,1 | A | | | Adult | | | | | 11,67 - 9,9 | 4,43 - 3,44 |

the passing of the days, the minimum for the last samples is 7 mm. The same goes for maximums, which varied from 7 mm for the first days to 10 mm at the end of a normal development process (ADD(-B10) for pupae *ad libitum*: 131.9-205.3). Fig. 1 and Fig. 2 show the major differences in the size of puparia and adults between those developed on D5 and those developed on D7. Similarly, adults developed from the youngest pupae show lengths of 6.4 mm. (Table I, Fig. 3A), comparatively lower than those developed from larvae fed *ad libitum* (L: 9.2-11.2 mm., Table I, Table II, Fig. 3B).

Emerged adults obtained from D5 maggots were reared in chambers with a mixture of powered milk and sugar (1:1) as source of food. After two days, a piece of kidney was introduced as substrate for oviposition. Three days later the egg-laying occurred normally and emerged larvae continued the life cycle without any problem developing into normal-sized adults (9,9-11,67 mm.) when fed *ad libitum*. Maggots fed *ad libitum* developed into normal sized adults. Reproductive capability was confirmed for undersized adults reared under starvation conditions. In a big extent, adult size depends on food availability during larval development. Similar starvation experiments done in October 2005 (Table I) and May 2006 (Table II) produced similar results that confirmed the adequate development of these specimens once they have moulted into third instar.

## Discussion

*Calliphora vicina* is a frequent studied species. Overspread, easy to be collected and to be reared under laboratory conditions, is usually selected as experimental model for developmental research. Diapause is induced under low tempeartures (11ºC) and absolute darkness (DD) in Scotland (Saunders, 1997). Nevertheless, we observed no diapause during winter months, with 9:15 LD periods and temperatures above 15 ºC. This species is recorded during winter months and considered a primary coloniser, dominant in cooler months, both in southern Europe and in northern Africa (Arnaldos *et al*., 2005; Tantawi *et al*., 1996).

Previous studies on the dispersal tendencies of Calliphoridae maggots during the postfeeding stage show *C.vicina* as a major dispersive species (Greenberg, 1990). Nevertheless, we observe in our cultures that this species

ususally pupates whether or not a specific substrate is laid out for it, with no special tendency to abandon food substrate or the rearing container during the postfeeding period (unpublished data). They even pupate on the organic matter source, which may be the corpse and clothes of the deceased. By contrast, other Calliphoridae species reared in our laboratory require more specific conditions for pupation, never pupate in areas close to the food source and die after a few days if no adequate substrate is found for pupation. This fact is especially observed in *Lucilia* s.l. species, including *L. sericata* (unpublished data). For the moment, we have not obtained good results in extreme situations for greenbottle fly species such as those referred to in the paper for *C. vicina.* Once again we observe clear differences in the biology of a relatively well studied species from one country to another. Biogeographical differences need to be adequately checked before extrapolating results from populations collected in a country to another.

Results obtained after starvation conditions show how 1st and 2nd instars need more time and food resources to develop adequately and accumulate the minimum amount of energy needed to confront pupariation, whereas recently moulted 3d instars accumulate enough energy to pupariate and evolve into gravid adults. Length and width measurements of individuals fed *ad libitum* are significantly greater than those from individuals under starvation condition (Fig. 3). Pupariation time is also longer *ad libitum* conditions, two more days exactly, probably due to the more quantity of biomass stored; that means a longer period and a bigger development of the adults emerged.

When starvation came sooner (D3), larvae (L1, L2) failed to reach the pupate instar and died. One reached that instar but was unable to emerge (first and second days of starvation, autumn experiment). In the following days larvae that reached the pupariation emerged successfully.

## Conclusions

*C.vicina* is a widely distributed species that arrives within the first few minutes after death and begins to lay eggs if environmental conditions are adequate for larval development. Maggots can pupate whether they have a specific substrate or not. They even pupate on the organic matter source. Starvation can induce premature pupariation at least in this species, but only when maggots are at the third instar. This may affect larval development times, which are clearly shortened when the food source is gone, and small adults are obtained if no more food can be found by the maggots. This fact is important when death occurs in an area where no more soft tissues can be found by the maggots, such as inside closed rooms, cars or containers once the corpse is intentionally removed. Applying the experimental results to a potential mummification process developed in ancient Egypt, similar results may be expected when maggots are removed from a corpse during the cleaning and evisceration process prior to mummification. Nevertheless, this is a first approach to a problem developed under laboratory conditions that clearly needs more research involving other species of forensic interest. Environmental conditions close to those inside Egyptian temples must be simulated in order to draw more precise conclusions.

Adults emerged from small puparia reared under starvation conditions are adequately developed and able to reproduce sexually, and could have maintained stable populations inside close environments such as temples in the Ancient Egypt, where corpses were mummified.

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