External factors and ecdysone release in *Calliphora erythrocephala*

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Summary. Ecdysone level is measured in the haemolymph of normal larvae and of larvae submitted to external stimulation. External stimulation delays the release of ecdysone and the formation of puparium. The adaptative value of the mechanism which connects the ecdysone titre to external stimulation is discussed.

Several physical or chemical factors disturb or delay formation of puparium (pupariation) of the Diptera: light, cold, heat[1], contact with water[2,3], tight but not constricting loops of cotton thread and inhibitors of aromatic amino acid decarboxylases[4]. Some of them temporarily inhibit the expression of hormone[5], whereas others oppose or prevent ecdysone release[4,6]. Unfortunately we have only indirect proofs about these mechanisms as the ecdysone titre in the animals submitted to these experimental conditions is unknown. It is therefore of interest to measure, with radioimmunoassay techniques, and to compare the ecdysone level in the haemolymph of normal larvae of *Calliphora erythrocephala* and of larvae submitted to an external stimulation which delays the time of pupariation.

The delayed pupariation. Larvae emerging from the same lot of eggs were divided into 2 batches at 5 days, when they leave the liver. Figure 1,A represents a batch of 25 isolated animals. Each larva was kept separate in a small Petri dish (6 cm in diameter) and covered with a small volume of saw-dust. In batch B, 25 identical larvae were maintained together in a single open Petri dish without saw-dust. The isolated animals rapidly cease movement. They remain motionless in the saw-dust and pupariate at 143.17±2.23 h. On the contrary, crowded animals (batch B) move continuously for the 2 days after leaving the food. They pupariate at 165.17±3.92 h.

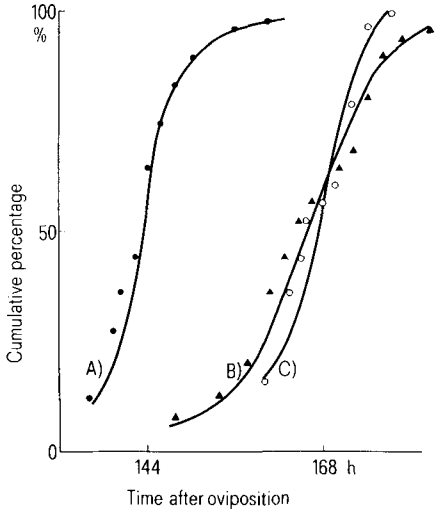

Fig. 1. Cumulative percentage of pupae. *A* Isolated animals; *B* larvae maintained together in a small volume; *C* isolated animals in a rolling round bottomed flask.

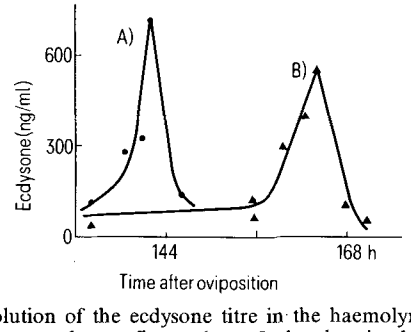

Fig. 2. Evolution of the ecdysone titre in the haemolymph during the experiment shown figure 1. *A* Isolated animals; *B* larvae maintained together in a small volume.

It has been shown[7] that the emergence of pupae from lightly spin-up last stage larvae of *Galleria mellonella* can be delayed by depriving the larvae of sufficient free space. The free space is probably not the main factor that modifies the time of pupariation in our experiment on *Calliphora*: a few larvae placed together with saw-dust in a round bottomed flask (15 cm in diameter) smoothly rolling on a horizontal axis (a Büchi rotovapor was used for this purpose) pupariate at 167.62±2.35 h (C in figure 1). The difference between the pupariation time in batches A and C or A and B is highly significant ($p < 10^{-9}$); the difference between B and C is not significant ($p < 0.3$): external stimulation delays the pupariation time as crowded conditions do.

The ecdysone titre. We measured the ecdysone titre in the haemolymph of motionless larvae A and of moving larvae B. Haemolymph was collected in 10-µl micro-pipettes and stored in 1 ml of methanol at 4 °C. The ecdysone determination was by radioimmunoassay[8]. In both batches A and B, the ecdysone level develops normally[9] and the peak occurs at the time of pupariation (figure 2): in batch A it is reached 22 h earlier than in batch B. From these experiments it appears that the initiation of pupariation of *Calliphora* depends upon external signals which control the release of ecdysone as moisture does in *Sarcophaga*[3].

We can assume that the inhibition of ecdysone release and pupariation by external stimulation allows the larvae to escape to unfavorable situations. When animals leave the food for a drier environment in the soil they usually cannot survive unprotected on the ground. It would be a distinct advantage for them not to turn immediately into the immobile puparium but to continue moving and to have inhibited ecdysone release until they find safe situations for pupariation. The mechanism by which ecdysone release relates to external conditions might have an adaptative value for the animal survival.

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