# Pheromones of the housefly

Noorman, N

# Chapter 7

## THE PREFERENCE FOR OVIPOSITION SITES AND THE PRESENCE OF AN OVIPOSITION PHEROMONE IN THE HOUSE FLY

**Abstract**
Houseflies prefer to deposit their eggs in clusters at one and the same site in crevices in rough substrates, especially when these have a smell of decaying animal products, such as fly eggs or dead flies. Clustering of eggs is not induced by the physical presence of the eggs. We found strong indications that an oviposition pheromone is deposited together with the eggs. This pheromone appears to disappear within a short period of time after oviposition either because it is very volatile or because it disintegrates.

## Introduction

Laying eggs in clusters is known to occur in several insect species. Eggs of egg-clustering butterflies are often aposematically coloured and distasteful to vertebrate predators. Depositing eggs in clusters may be of advantage to the eggs, the larvae as well as the adults (Stamp, 1980). Egg clustering decreases the exposed surface. It thus reduces desiccation and the accessibility to parasitoids and predators. In addition, larvae that feed, rest and moult synchronously may grow faster than those that live singly, and also the protection from parasites and predators may be better than when larvae stay apart. Aggregated larvae were shown to be four times as active than solitary larvae, spent 25 % more time feeding, had a higher fat content, and pupated sooner (Long, 1953). Finally, the adults have to spend less time to find mates and to search for suitable oviposition sites when they are close together than when they live widely separated.

In several dipteran species it has been found that females aggregate at oviposition sites. In the blackfly *Simulium damnosum* the phenomenon of aggregated oviposition was investigated under controlled laboratory conditions (McCall *et al.*, 1994, 1995). It appeared that significantly more females oviposited on substrates already containing eggs or volatiles from freshly laid eggs than on control substrates without these substances. Moreover, the females landed more quickly when more eggs were present on the substrate. The authors suggested that specific attractants were emanating from freshly laid eggs.

In the sandfly *Lutzomyia longipalpis,* Elnaiem and Ward (1990) found that the presence of eggs resulted in higher oviposition rates than when eggs were absent. Eggs washed in hexane and water did not induce oviposition indicating that the positive response was due to chemicals present on the eggs (Elnaiem and Ward, 1991). In addition, Dougherty *et al.* (1994) showed that chemicals extracted with hexane from eggs of *L. longipalpis* attracted gravid females of this species for oviposition. The females oviposited earlier and laid more eggs on substances containing these chemicals than on substances on which these substances were absent. Dougherty and Hamilton (1997) identified dodecanoic acid as the oviposition pheromone of the sandfly.

Females of *Culex* spp. lay their eggs in boat-shaped clusters on the surface of stagnant, organically rich water. The egg rafts attract gravid females of *C. tarsalis, C. pipiens molestus* and *C. quinquefasciatus* and lead them to lay at the same sites (McCall and Cameron, 1995). Dawson *et al.* (1989) showed that eggs of *C. quinquefasciatus* emit a volatile pheromone that induces gravid females to oviposit near freshly laid eggs.

In this study we examined the possible presence of an oviposition-stimulating chemical in *Musca domestica*. Aggregation of insects during oviposition gives the opportunity to catch gravid females and thus to reduce the numbers of insect in a population drastically.

## Materials and methods

*Insects*

Experiments were done with *Musca domestica* L. flies from the laboratory strain WHO Ij2 1961, which was obtained from the Statens Skadedyrlaboratorium, Lyngby (Denmark). The flies, about 300 per cage, were reared in cages (30 x 30 x 40 cm) in a L:D 12:12h regime at 25 °C and r.h. 70%. Light was provided by 2 white fluorescent tubes (Philips TL 40W/33). The flies were fed (ad libitum) a mixture of sugar, powdered milk and yeast (5 : 5 : 1 by weight). In addition, a fountain filled with tap water was present. A mixture of yeast, powdered milk, agar and water (5:5:1:25 by weight) was used as ovipostion substrate and larval food.

*Bioassay*

All experiments were carried out in rearing cages in the culture chamber. In a preliminary series of experiments 4 oviposition substrates were offered, simultaneously in one cage, in Petri dishes (5 cm i.d.) containing (respectively) wet smooth filter paper impregnated with fly food, wet grains of kilned clay (3 mm diam.) impregnated with fly food, a smooth layer of larval food, and a rough layer of larval food.

In the subsequent experiments oviposition occurred in glass tubes (7 cm high, 1.5 cm i.d.) filled with clay grains (3 mm diam.) and tap water except for the upper 2 cm which contained a folded strip of filter paper (3 mm wide, 30 cm long); the lower 7-cm-long end of the filter paper extended to the bottom of the tube. The folded paper, which remained humid by capillary attraction, was offered as oviposition substrate either without or with additional stimuli (see Results section).

Estimation of the number of eggs deposited was done by putting the eggs in 1 litre tap water. After thorough shaking, 4 samples of 1 ml of the mixture were taken in which the eggs were counted. An estimation of the total number of eggs was made by multiplying the average number of eggs present in the samples by 1000. The number of females which had been ovipositing was calculated by dividing the number of eggs by 85 (observed average number of eggs deposited by 1 female during oviposition).

## Results

*Preliminary experiments*

Two experiments were done on successive days using the same flies. Table 1 shows the results of these studies. By far the most eggs were deposited on clay grains and rough larval food. About 15 females used these substrates as oviposition sites on the first day and about 80 females on the second day. Smooth filter paper and smooth larval food were far less attractive to the females. Only 3 females deposited their eggs on these substrates. The results suggest that the females strongly prefer to lay their eggs on substrates with crevices above smooth substrates. Therefore, the oviposition experiments were continued with wet folded filter paper in glass tubes, as described in Material & Methods section.

Table 1. Number of eggs on different substrates.

| | Fly's food on | | Larval food | |
|---|---|---|---|---|
| | Smooth paper | clay grains | smooth | rough |
| Day 1 | 0 | 300 | 90 | 1000 |
| Day 2 | 80 | 5000 | 100 | 2000 |

*Filter paper experiments*

Experiment 1:

Flies were offered, during one day, 5 standard tubes containing wet filter paper without additional substrates. The next day 5 fresh tubes were placed in the same cage. The tubes were randomly distributed over the bottom of a cage. It appeared that on both days all eggs were deposited on the filter paper in one and the same tube. Which tube was chosen was determined by the female that started to oviposit first, and this did not depend on the position of the tube in the cage. On the first day about 80 females laid 6750 eggs in one tube, and on the second day 120 females deposited 10,000 eggs in a tube at another place on the cage's bottom.

Experiment 2:

In this experiment the effects of the presence of fresh and old housefly eggs and of the smell of the latter on oviposition in the tubes was tested. Flies in one cage were offered 8 tubes of which one was loaded with fresh eggs (2-6 h old). This experiment was repeated 3 times with the same flies on successive days. In another cage 8 tubes were placed of which 1 contained old eggs (3 days old, decaying). This experiment was repeated the next day. In a third cage, during one day, 8 tubes were present of which one contained the smell of old eggs (eggs had been present in the tube for 3 days).

Table 2 shows that visual or tactile stimuli from the eggs or the smell of fresh eggs did not stimulate gravid females to deposit their eggs in tubes where these stimuli were present. On the 3 successive days all females (about 150) laid their eggs in tubes were no fresh eggs were present. The general tendency of flies to lay their eggs at one and the same site is, however, unquestionable shown. However, when a tube contained old decaying eggs or only the smell of these, all females (about 325) except for 1 chose for the tubes with these stimuli.

Table 2. Number of eggs deposited in tubes with (shaded) and without stimulus.

| | Tubes | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Stimulus | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| Fresh eggs 1 | 0 | 0 | 0 | 0 | 5000 | 0 | 0 | 0 |
| Fresh eggs 2 | 0 | 0 | 0 | 0 | 0 | 0 | 85 | 0 |
| Fresh eggs 3 | 0 | 0 | 0 | 0 | 0 | 80 | 7500 | 0 |
| Old eggs 1 | 0 | 85 | 9500 | 0 | 0 | 0 | 0 | 0 |
| Old eggs 2 | 0 | 0 | 0 | 0 | 0 | 10000 | 0 | 0 |
| Smell old eggs | 0 | 0 | 0 | 0 | 8000 | 0 | 0 | 0 |

Experiment 3:

In this experiment the smell of 3-day-old dead flies was tested for its attractiveness to gravid females. To exclude visual or tactile stimuli 10 dead flies were placed in the test tube under the water surface between the clay grains. On two successive days 8 tubes (7 without, 1 with dead flies) were presented to flies in one and the same cage. On the first day about 95 females (8000 eggs) chose for the baited tube and only 1 female (75 eggs) oviposited in the unbaited one. On the second day about 4 flies (350 eggs) chose for the tube with stimulus and none for the unbaited tubes.

Experiment 4:

In this experiment larval food was added as oviposition substrate. The food was offered fresh (newly made) or 2 days after preparation when it had started to decay. Two experiments were carried out on 2 successive days with flies in one and the same cage. The results are shown in Table 3. On the first day about 20 females deposited their eggs in the tube with fresh larvae food, whereas about 90 females laid their eggs in a tube without this substrate. On the second day about 150 females chose for the tube with fresh larval food and none for the seven tubes without this substrate. Decaying larval food attracted about 120 females on the first day and no females on the second day. In the latter case 35 females deposited their eggs in a tube without substrate. The results do not indicate a preference for either fresh or old larval food. Again the clustering effect in egg deposition is unmistakable present.

Table 3. Number of eggs in tubes with (shaded) and without stimulus.

| | Tubes | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Stimulus | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| Fresh larval food 1 | 0 | 1800 | 0 | 0 | 0 | 0 | 7500 | 0 |
| Fresh larval food 2 | 0 | 0 | 0 | 0 | 0 | 13000 | 0 | 0 |
| Old larval food 1 | 0 | 0 | 0 | 10000 | 0 | 0 | 0 | 0 |
| Old larval food 2 | 0 | 3000 | 0 | 0 | 0 | 0 | 0 | 0 |

Experiment 5:

To determine whether egg clustering would also occur when more tubes with an attractive stimulus were offered, 3 experiments on 3 successive days with the same population of flies were done. Each day 10 tubes, 5 baited with dead flies (in the same way as in experiment 3) and 5 unbaited, were placed randomly on the bottom of the cage. The results are presented in Table 4. On the first day most of the females were not yet ready to oviposit but the few females that laid eggs deposited them in 3 out of the 5 tubes baited with dead flies. On the second day about 60 females deposited their eggs in only one tube baited with dead flies. On the third day about 80 females chose for one and the same baited tube whereas only 1 female oviposited in a tube without dead flies.

Table 4. Number of eggs in tubes with (shaded) and without stimulus (dead flies).

| | Tubes | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| Day 1 | 0 | 0 | 0 | 0 | 200 | 0 | 0 | 70 | 60 | 0 |
| Day 2 | 0 | 0 | 5200 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Day 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 7000 | 50 | 0 |

Experiment 6:

The presence of eggs 2–6 hours old did not enhance oviposition (Experiment 2). This suggested that, if an oviposition stimulating pheromone is deposited together with the eggs, this pheromone may be very volatile or may disintegrate within a short period of time after oviposition. Therefore, in a final experiment we investigated whether the smell of very fresh eggs may attract gravid females.

In 3 different cages one oviposition tube was placed, which was removed within 30 minutes after egg deposition in the tube had started. About 1000 eggs were collected from each tube, put into 3 new tubes and covered with the humid filter paper strip in such a way that the flies could not notice the physical presence of the eggs. Then, in each of the 3 cages one of these new tubes was placed together with 4 unbaited ones; the 5 tubes were randomly distributed over the bottom of the cages. It appeared that oviposition immediately started again and without exception all eggs were laid in the tubes with the fresh eggs.

**Discussion**

The described experiments clearly demonstarte that gravid females have a strong drive to lay their eggs in clusters at one and the same site and in crevices on rough substrates, even when no additional visual or chemical stimuli are present. Smooth surfaces do not seem to be a suitable place for females to deposit eggs. The reason for this may be that eggs laid in crevices and in clusters are more protected against desiccation and predation. Moreover, as already said before, clustering of eggs may have several advantages resulting in higher survival rates for the eggs, larvae as well as the adults. We also found strong indications that the clustering strategy secures survival of the fittest when temporary food shortage occurs. In places with low availability of food, eggs and young larvae may serve as a food source for older larvae. In fact, we frequently observed that without additional food source a number of larvae in a cluster survived and pupated by eating eggs and other larvae.

The smell of decaying organic material attracts gravid females and induces oviposition, probably because these substrates may be suitable food sources for the larvae. In addition, we have strong indications that the odour of eggs collected within 30 minutes after oviposition is attractive to gravid females and stimulates ovipostion, suggesting the presence of an oviposition stimulating pheromone. However, 2-6 h old eggs do not enhance oviposition, suggesting that the pheromone may disappear shortly after oviposition. This agrees with the results of McCall (1995), who found that significantly more blackflies (*Simulium damnosum*) oviposited on substrates baited with freshly laid eggs than on control substrates. Substrates baited with 12-hour-old eggs were not significantly more attractive

Application of an oviposition stimulating pheromone at suitable locations in the flies' habitat provides new perspectives to environmentally friendly control of houseflies. The possibility to control flies at moments within their life cycle when new generations are created would provide a most effective way of attacking the problem, namely the nuisance of the flies. Removal of individuals from the population at a moment they do not cause annoyance and are not yet able to propagate is a more efficient way of control than removal of adult individuals. The challenge of further is to identify the oviposition pheromone.

**Acknowledgements**

We thank Dr J. B. Jespersen of the Danish Pest Infestation Laboratory, Lyngby (Denmark), for providing us with pupae of the WHO Ij2 strain.

This research was supported by the Technology Foundation (STW).