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# A new method for the study of feeding behavior in the fly *Ceratitis capitata*

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A simple new method for the study of feeding behavior in the fly *Ceratitis capitata* is presented, consisting of 22 h of food deprivation and 2 h of food availability. During the latter period the number of flies feeding is counted every 5 min. Results showed that the animals learned to ingest all the food they needed during the 2 h, with a feeding pattern in which the number of flies feeding on a white diet increased in the beginning of the 2-h period and gradually decreased towards the end of it. They also showed that the flies demonstrated a preference for feeding on a red diet (with the same pattern as for the white diet) rather than on a green diet (which did not result in the feeding pattern of an increased number of flies feeding at the beginning of the session and a decreased number at the end). The data suggest this method can be used in a variety of situations in which ingestion is involved.

The Mediterranean fruit fly *Ceratitis capitata*, found worldwide, is one of the most destructive insect pests for agriculture. There have been investigations of its innate behaviors, such as courtship and mating (e.g., Carey, Krainacker, & Vargas, 1986; Prokopy & Hendrichs, 1979; Webb, Calkins, Chambers, Schwienbacher, & Russ, 1983), or food selection (e.g., Zucoloto, 1987), but the learned aspects of the behavior of *C. capitata* have until now received little attention—in contrast to what has been done with respect to learning in other insects, such as, to mention only a few, grasshoppers (e.g., Bernays & Wrubel, 1985), or the related *Drosophila* (e.g., Le Bourg, 1987; Tempel, Bonini, Dawson, & Quinn, 1983), or even bees (e.g., Pessotti, 1972, 1981; Pessotti & Otero, 1981; Pessotti & Sénéchal, 1981a, 1981b). One exception, however, is a study demonstrating that *C. capitata* females learn to accept or refuse a particular host fruit type for oviposition (Cooley, Prokopy, McDonald, & Wong, 1986).

In an attempt to investigate learning in the fruit fly, we isolated adult flies and deprived them of food but not water, in order to provide something with which to reward them and to study the learning of an arbitrarily chosen behavior. To our surprise, and contrary to what happens with other laboratory animals such as rats, isolated 22-h deprived flies did not feed when exposed to food for 2 h. Increasing this period for up to 4 h (in some cases for more than 6 h) also did not provoke any feeding episodes. This was in clear contrast with nondeprived flies kept in groups in rearing cages, where, at any moment, it was possible to observe flies feeding on the diet or water. This raised the hypothesis that being grouped is important for feeding.

The present study was aimed at developing a method for the study of feeding behavior among food-deprived grouped flies. We also intended to investigate the method's usefulness for studying other topics such as preference for diet color.

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## METHOD

### Subjects

Recently born adult flies separated according to sex and kept in groups of 25 (except for one group of females) at 24-26 °C were studied until they died. The experiments were carried out during the months of May through July.

### Apparatus

The flies were housed in 25×25×25 cm tulle-walled cages with water permanently available in soaked pieces of cotton. When not restricted, food was placed on top of 6-cm high plastic cylinders (4 cm in diameter) of a milky white color, which were placed inside the cages through a sleeve serving as a door in one of the walls.

### Diet

The composition of the diet was 15 g corn starch, 6 g sucrose, and 100 ml tap water. Such a diet can be stored in the refrigerator and used for 10-15 days. The components were cooked in a hot bath for 3-5 min and then put in petri dishes kept covered in the refrigerator until used. One advantage of this diet is that it is white, very much like the cylinders the food was put on top of, and it can easily be colored by the addition of dietary dyes in amounts appropriate for the current experiment. On this diet, some of the flies lived up to more than 60 days.

### Dyes

Dietary red and green powdered dyes (Arco Iris Brasil[R]) were dissolved in distilled water (2.5 g/20 ml). For each 100 ml of water, 3.5 ml of the dye solution was used.

### Procedure

The flies were separated according to sex on the day of birth and put in the cages with water ad lib but no food. From the next day on, until

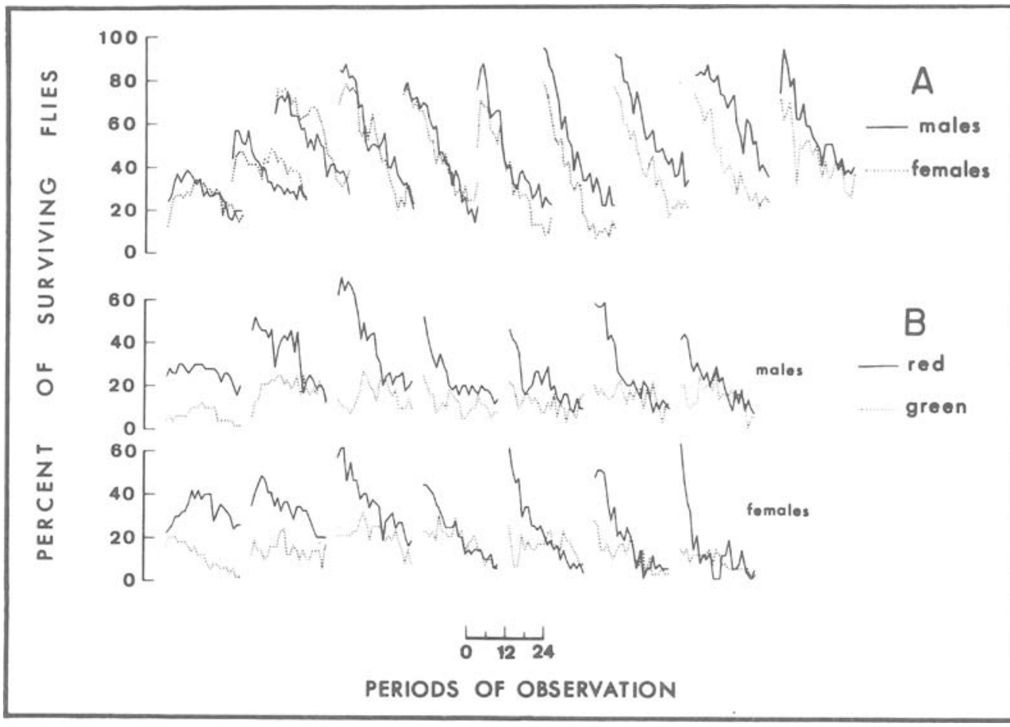


**Figure 1. Number of flies feeding at the end of each 5-min period of observation. In the ordinate, number of flies feeding expressed in percent of surviving flies in all cages. In the abscissa, successive 5-min periods in which the flies were counted. (A) white diet. (B) red/green diet.**

the day the last fly died, four plastic cylinders (with four pieces of diet placed equidistant from each other) were put near the corners inside each cage at 1:30 p.m. and removed 2 h later. During this period, the number of flies with the proboscidis in contact with the diet was counted at every 5 min. Thus each session yielded 24 counts, which were pooled and transformed into a percentage of the surviving flies in all cages.

## RESULTS AND DISCUSSION

Figure 1A shows the number of flies feeding (i.e., with the proboscidis in contact with the white-colored diet) in each 5-min period, from the 1st to the 10th day of observation. The remaining data are not shown, because the feeding pattern did not differ significantly; but the number of flies feeding continued to be recorded until the day the last fly died. It may be seen that on the 1st day, not more than 40% of the flies were observed to feed, and only during some periods of observation. From the 2nd day on, there was an increasing tendency to feed as soon as the diet was made available. The percent of flies feeding was greater at the beginning of each session, gradually and regularly decreasing toward its end. This may be interpreted to mean that the flies learned to ingest all the food they needed during the daily 2-h period of food availability. In contrast, in a cage with food ad lib, it is possible to find flies feeding all day long. It may be argued that this model does not depict learning but simply selects flies that feed during the availability period from the 1st day on and lets the others die from starving during the remaining 22 h of deprivation. This seems not to be the case, however, because the number of flies dying in the first 4–6 days was very small (Table 1) and cannot account for the percent increases in the number of flies feeding at the beginning of each session. One disadvantage of this method is that it may be difficult to observe and record for individual flies. Nevertheless, it is a simple method that can be applied to a variety of situations, such as learning studies, preference experiments, and so forth. Thus, for example, we used the method in the following experiment, which was aimed at investigating whether the flies possess any innate preference for diet color.

**Table 1**
**Number of Surviving Flies on Consecutive Days**

| | White Diet | | Red/Green Diet | |
|---|---|---|---|---|
| Day | Males | Females | Males | Females |
| 1 | 75 | 70 | 50 | 50 |
| 2 | 75 | 70 | 48 | 49 |
| 3 | 75 | 69 | 48 | 47 |
| 4 | 75 | 66 | 48 | 47 |
| 5 | 75 | 66 | 47 | 46 |
| 6 | 69 | 64 | 47 | 46 |
| 7 | 69 | 63 | 47 | 45 |
| 8 | 69 | 63 | | |
| 9 | 66 | 62 | | |
| 10 | 60 | 62 | | |

Figure 1B shows the number of flies feeding in a situation similar to the one described for Figure 1A. The only exception was that instead of presenting the flies with four white cylinders with four pieces of white diet on each one, they were presented with two cylinders with four pieces of a red-colored diet on each one, as well as two others with the same amount of a green-colored diet.

It may be seen that the same feeding pattern that was shown for the white diet in Figure 1A arose here for the red diet but not for the green diet. In other words, the number of flies feeding on the red diet increased at the beginning of each session, while the number of flies feeding in the green diet remained more or less constant along the sessions. It must be said, however, that from the 4th–6th day on, some flies, after feeding on the red diet (which was easily recognizable because their abdomen became red 1 min after ingestion), went to feed on the green diet. In a few sessions (data not shown), in some of the periods, the number of flies feeding on the green diet was greater than the number feeding on the red diet. Such a preference for colors has been reported before. Souza, Pavan, & Silva (1984) demonstrated that red-colored agar spheres were more attractive for oviposition in comparison with green or yellow spheres. They also demonstrated that females preferred to sit in the red spheres, whereas males preferred the yellow ones as opposed to the green spheres. Our results support and extend these data on oviposition and alightment-color preference to a situation of preference for diet color. We did not, however, use more than two colors, so we are presently carrying out experiments testing others.

At present, many unanswered questions remain. What is being learned? Is it possible that disturbances in the cage (associated with the introduction of the diet) have become discriminative stimuli signalling the occasion for feeding? Or that other disturbances (associated with the removal of the diet) signal the absence of food? Or do the animals learn to recognize the diets as food? Why does the percent of flies feeding on the red diet increase at the beginning of each session (as is the case for the white diet, which we interpret as learning), whereas the percent feeding on the green diet remains as a, so to say, "background frequency"? Nonetheless, as we have already said, this is a very simple and easily prepared method (it is very inexpensive as well), which can be useful in a variety of situations, ranging from the study of innate preferences for food color to the investigation of parameters of learning, not to mention the study of the nutritional value of laboratory diets.

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